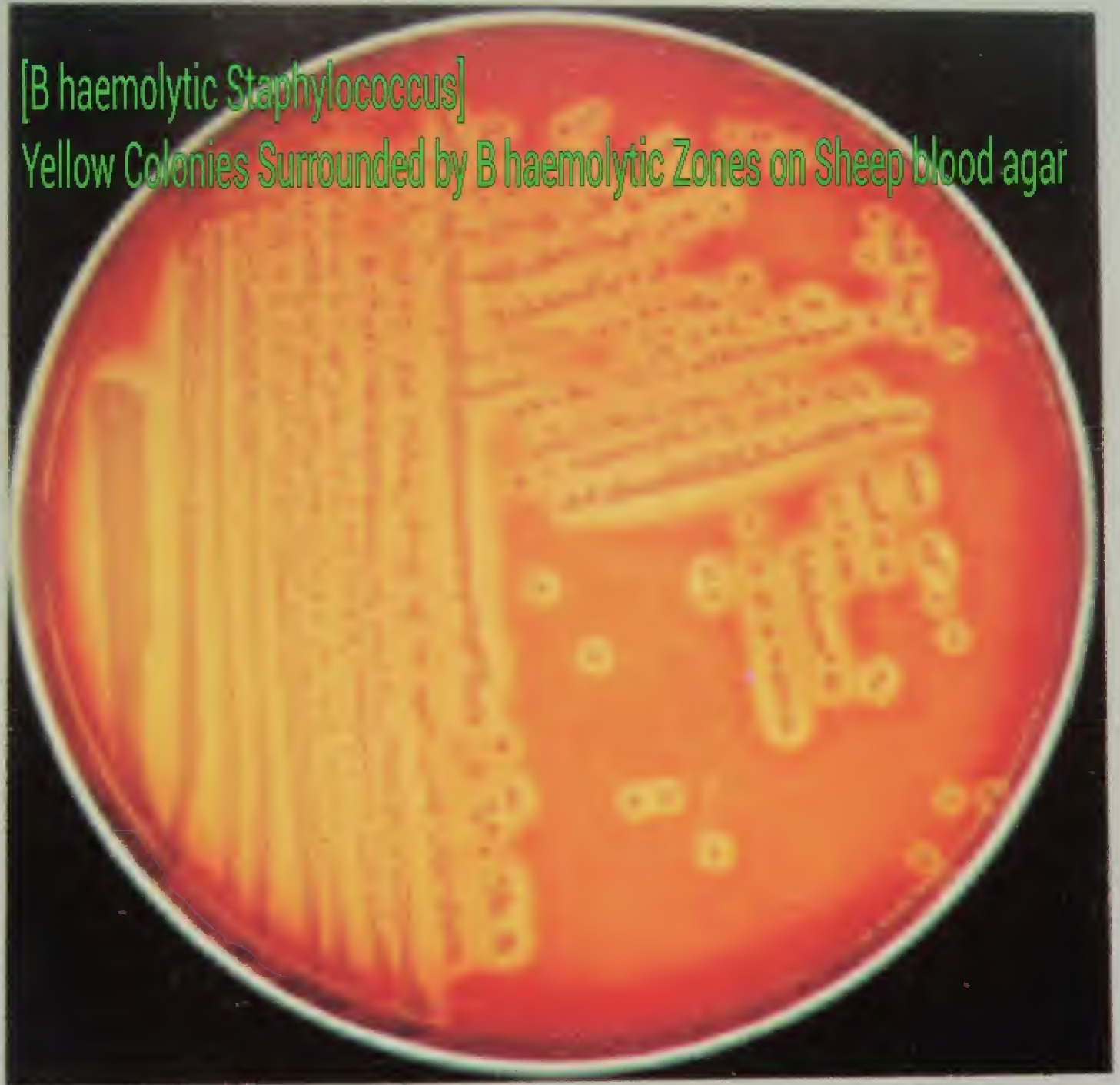


[Non haemolytic Staphylococcus]  
white non haemolytic Colonies On Sheep blood agar



[B haemolytic Staphylococcus]

Yellow Colonies Surrounded by B haemolytic Zones on Sheep blood agar





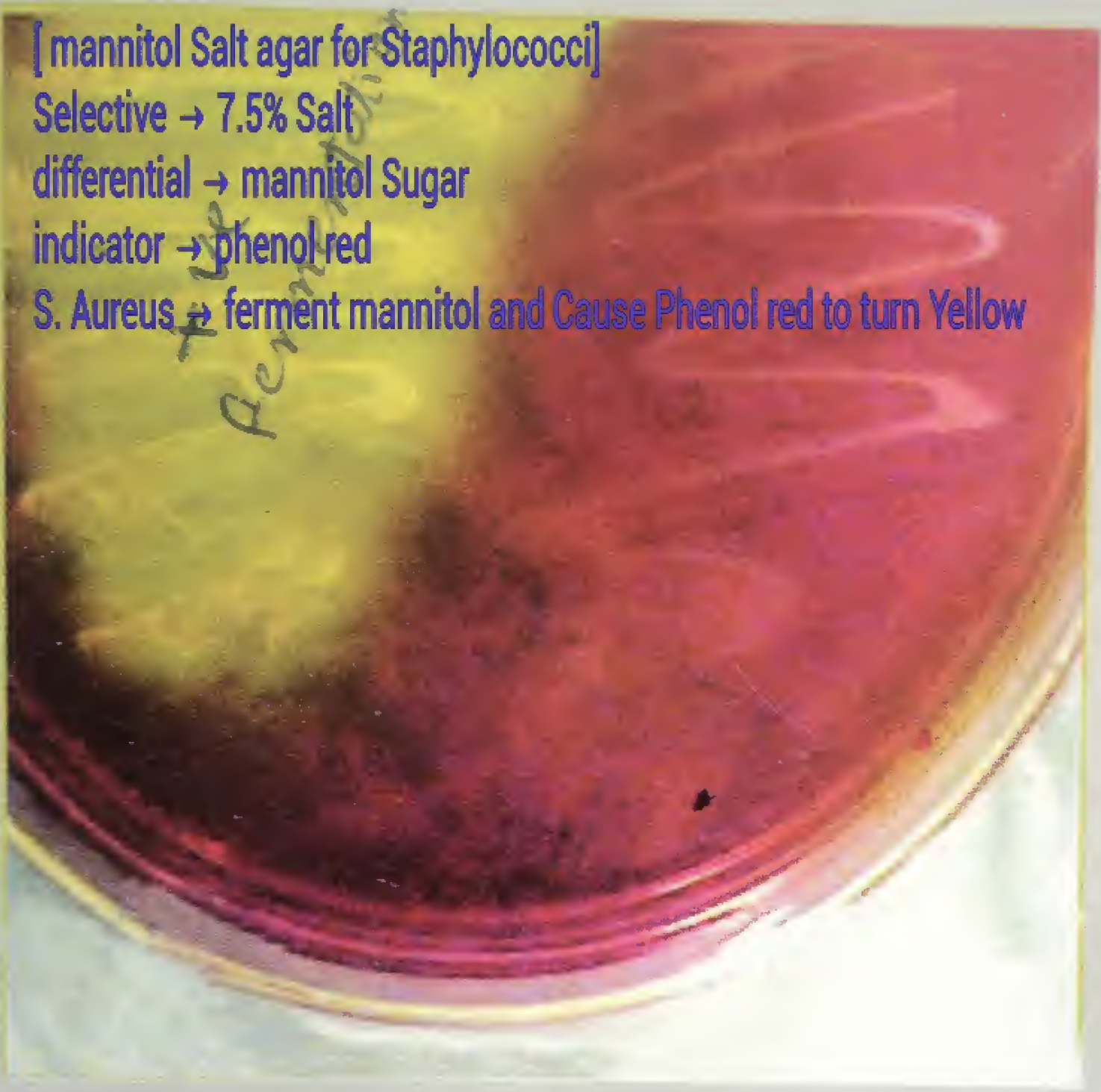
[mannitol Salt agar for Staphylococci]

Selective → 7.5% Salt

differential → mannitol Sugar

indicator → phenol red

S. Aureus → ferment mannitol and Cause Phenol red to turn Yellow



A petri dish containing a bacterial culture on a red agar medium. A streak of bacteria is visible, showing a zone of alpha haemolysis (opacity) and a zone of black precipitate (iron sulphide) at the bottom of the streak.

[*Streptococcus equi*]

alpha haemolysis

H<sub>2</sub>S production + iron → iron Sulphide [Black]

S, Viridans [ a haemolytic Streptococci ]

Colonies Surrounded by narrow Green Zones

H<sub>2</sub>O<sub>2</sub> + haemoglobin → met haemoglobin [ green ]





[ B - haemolytic Streptococci ]

pin point Colonies Surrounded by Sharply defined haemolytic areas



# [ Enterococcus agar for *E. fecalis* ]

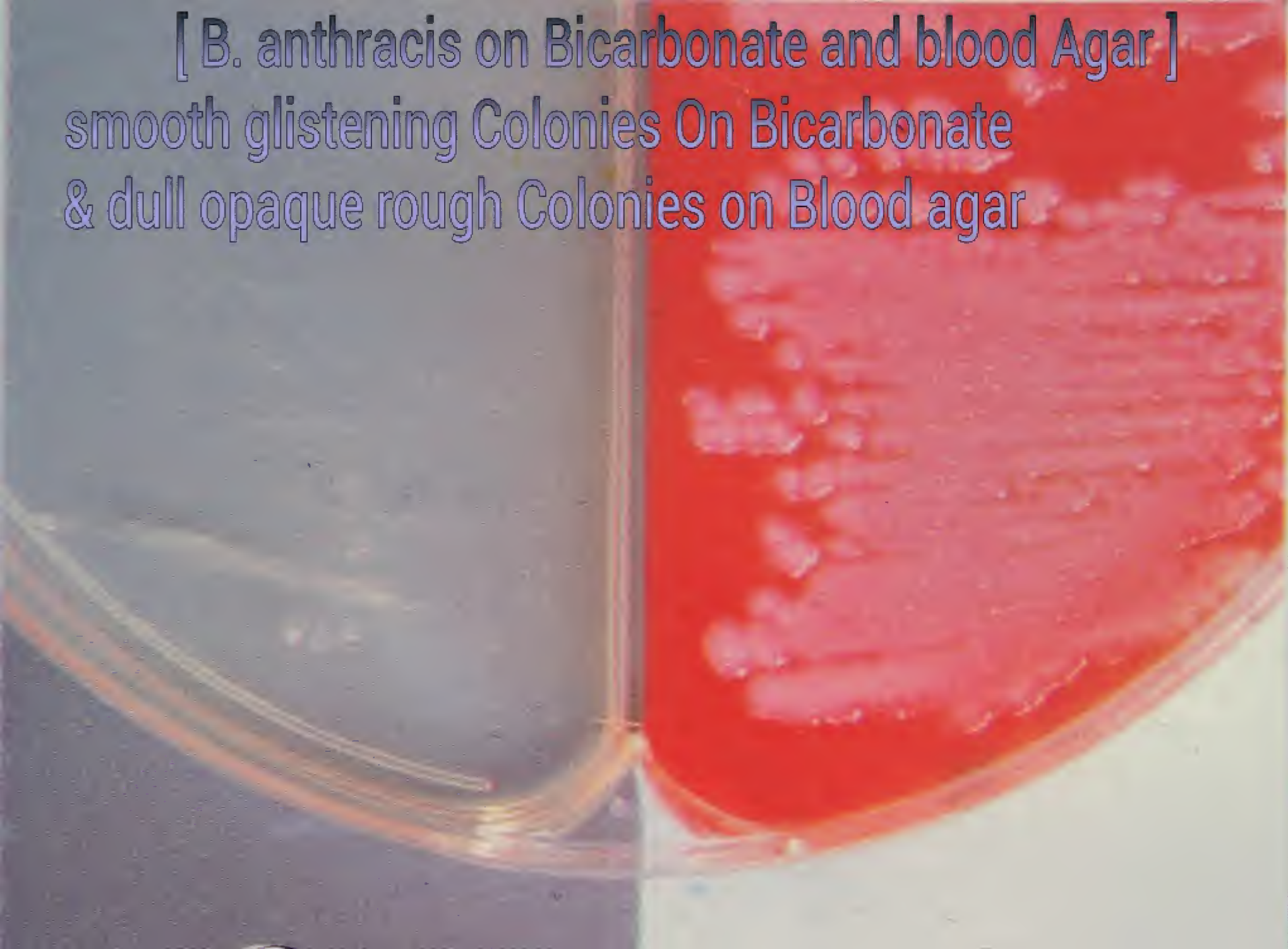
Contain Sodium azide → inhibit gram -ve bacteria  
tetrazolium chloride reduced to formazon [ red ]





[ *B. anthracis* on Bicarbonate and blood Agar ]

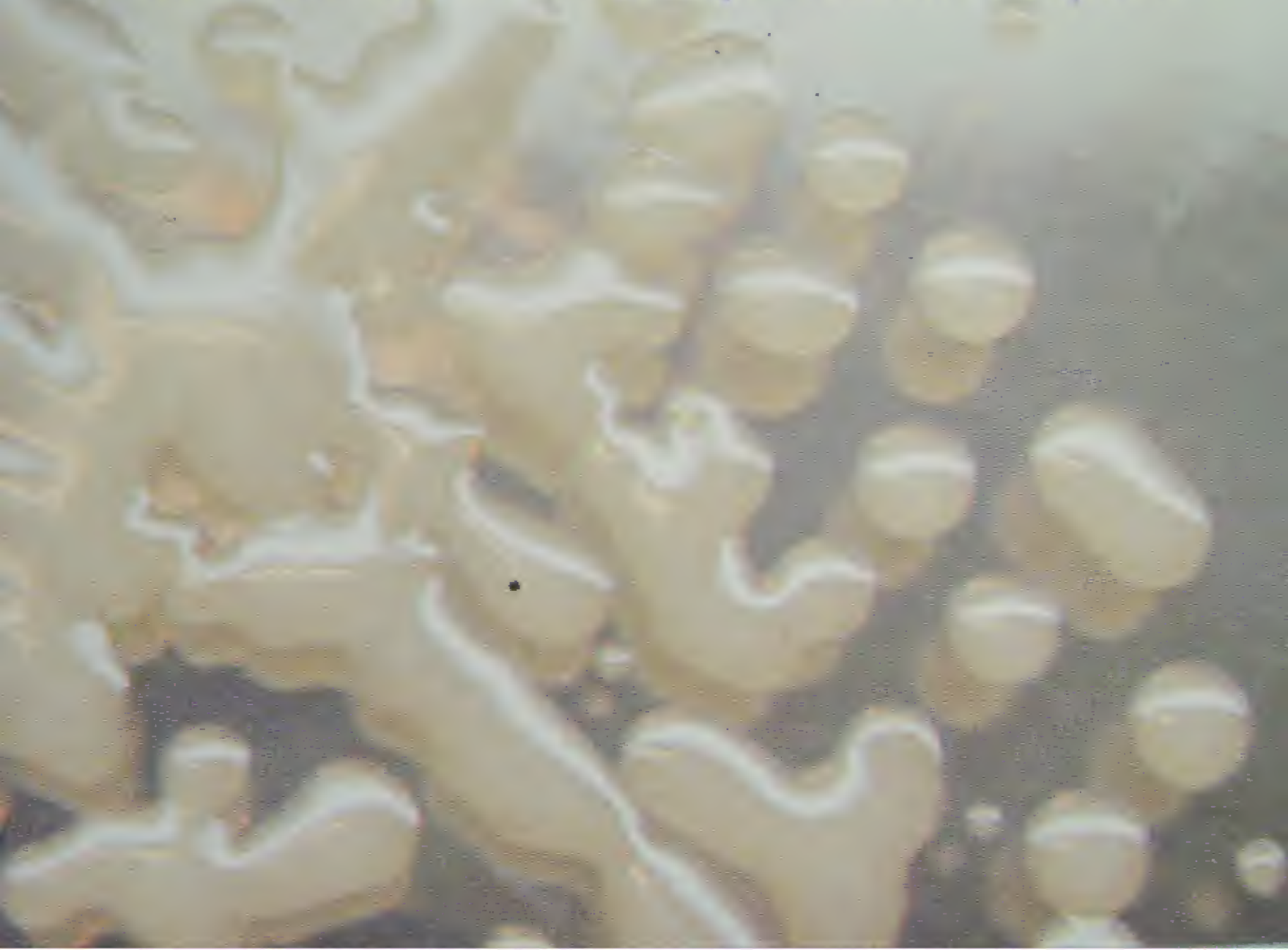
smooth glistening Colonies On Bicarbonate  
& dull opaque rough Colonies on Blood agar





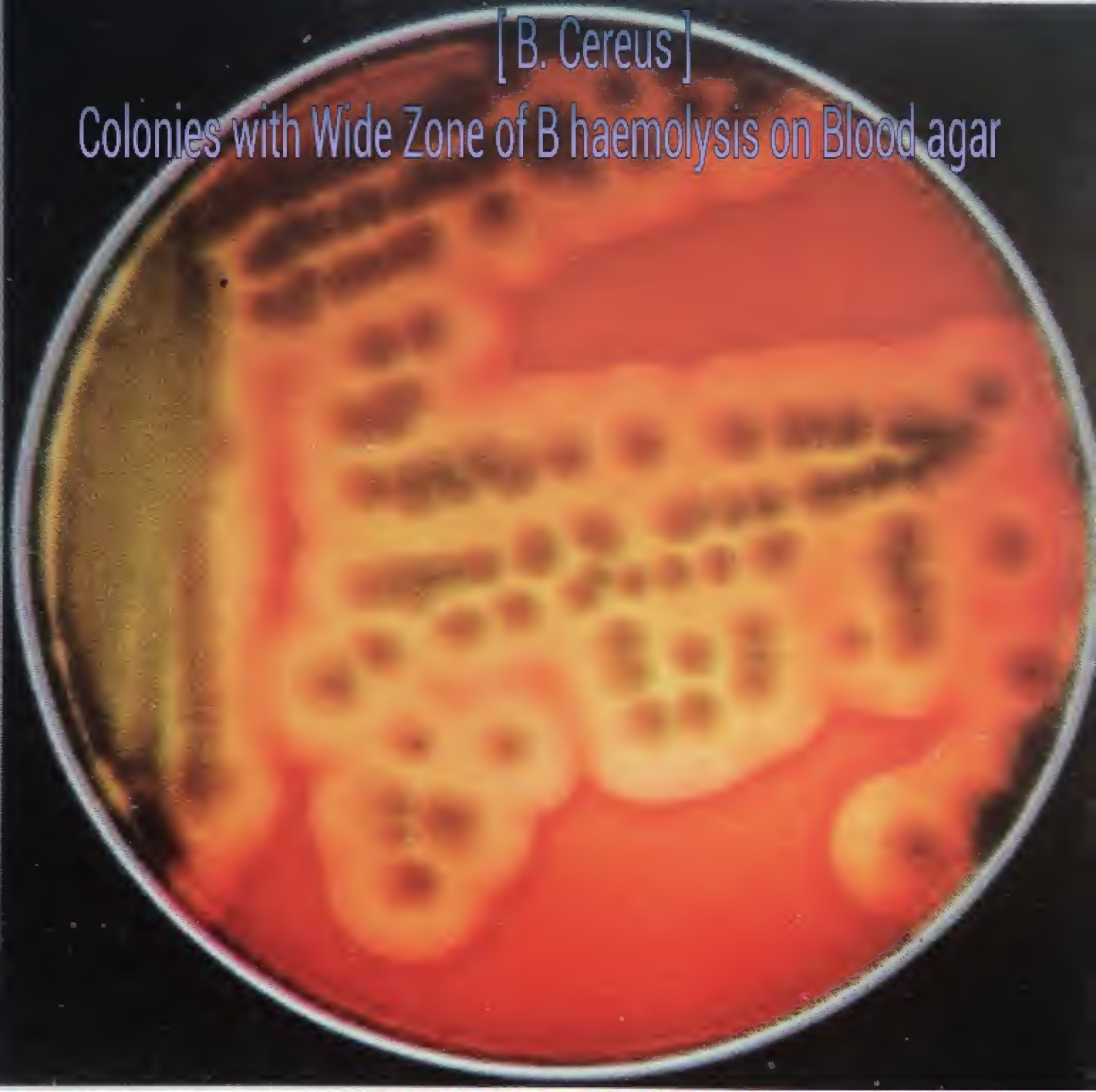
# [ *B. anthracis* Muroid Colonies ]

Muroid Colonies occur due to production of Capsule



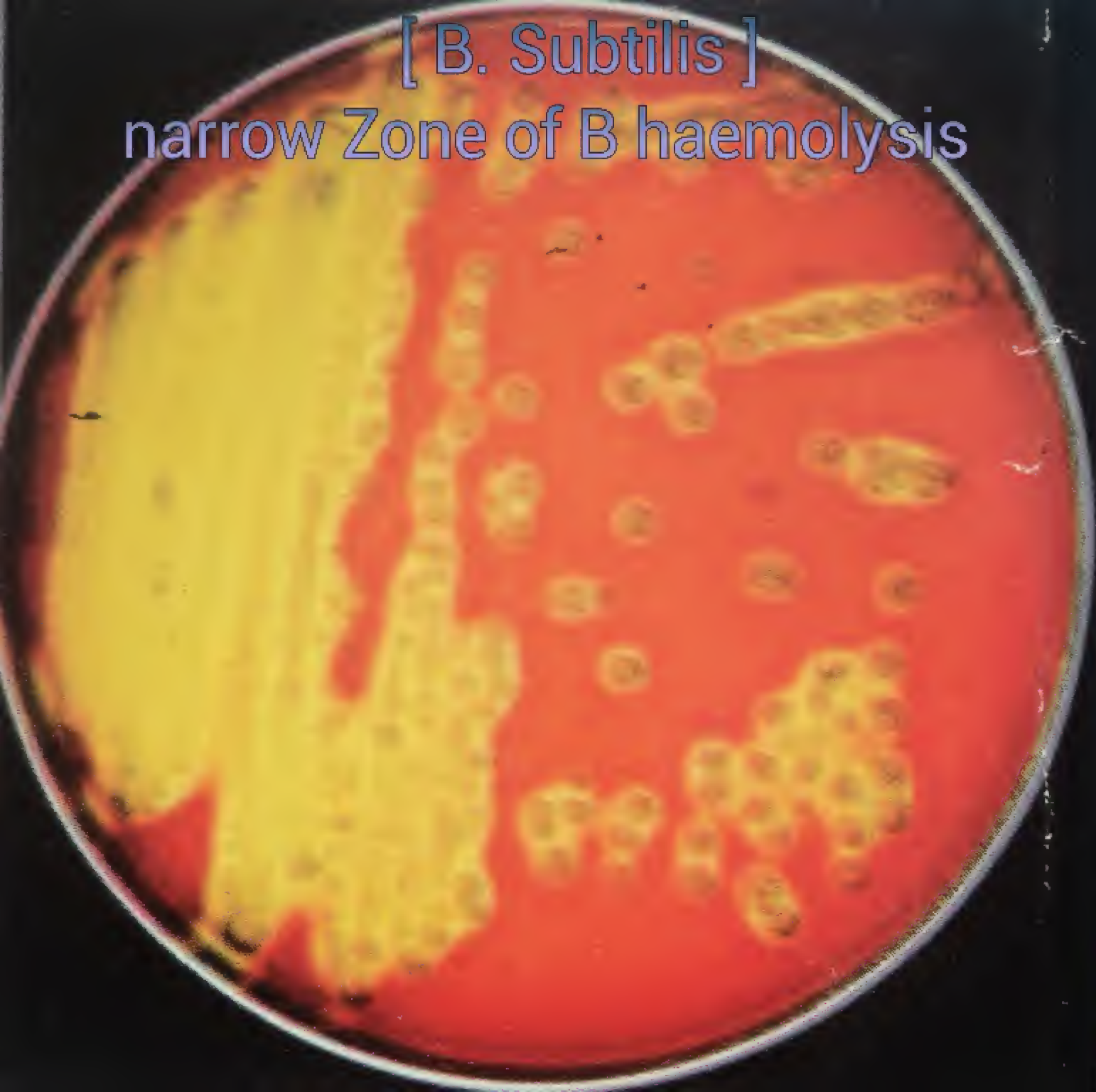
[ *B. Cereus* ]

Colonies with Wide Zone of  $\beta$  haemolysis on Blood agar





[ *B. Subtilis* ]  
narrow Zone of B haemolysis



**B. Cereus** [ BCSA → Bacillus Cereus Selective agar ]

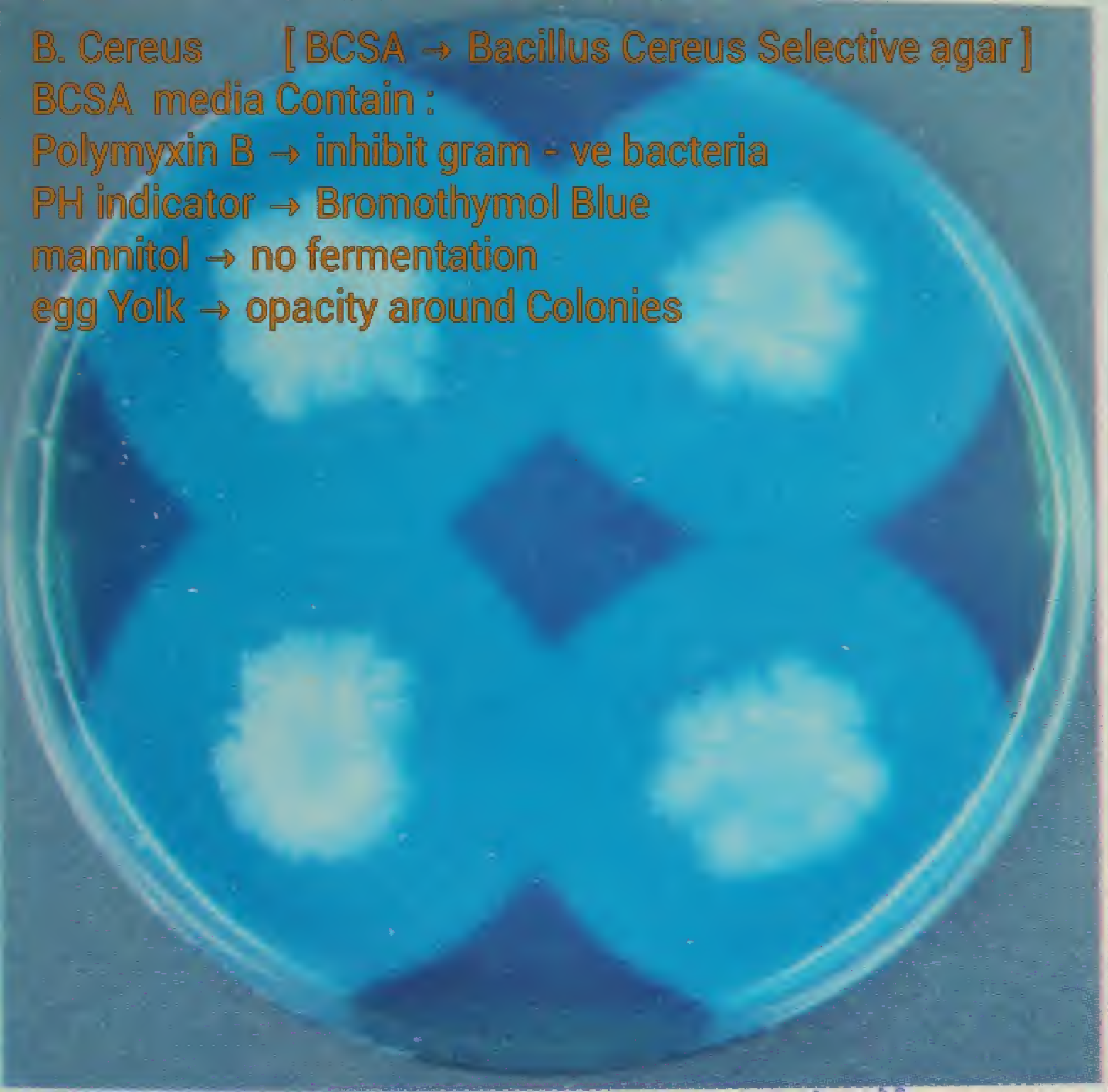
BCSA media Contain :

Polymyxin B → inhibit gram - ve bacteria

PH indicator → Bromothymol Blue

mannitol → no fermentation

egg Yolk → opacity around Colonies





**B. Subtilis [ BCSA → Bacillus Cereus Selective agar ]**

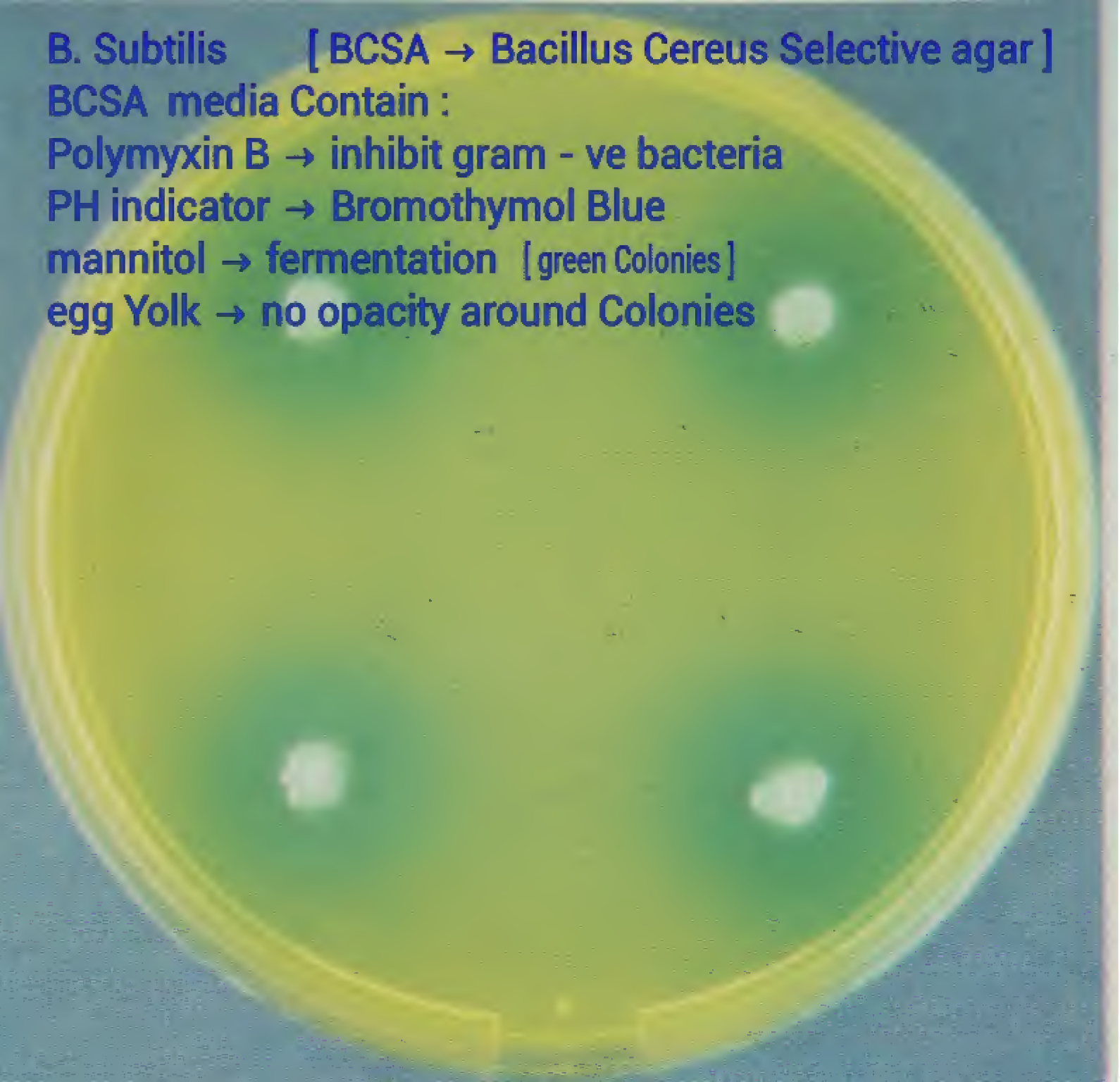
**BCSA media Contain :**

**Polymyxin B → inhibit gram - ve bacteria**

**PH indicator → Bromothymol Blue**

**mannitol → fermentation [ green Colonies ]**

**egg Yolk → no opacity around Colonies**



# [ *C. perfringens* On Blood agar ]

Colonies Surrounded by Two Zones of haemolysis

inner → Complete haemolysis due to Theta toxin release

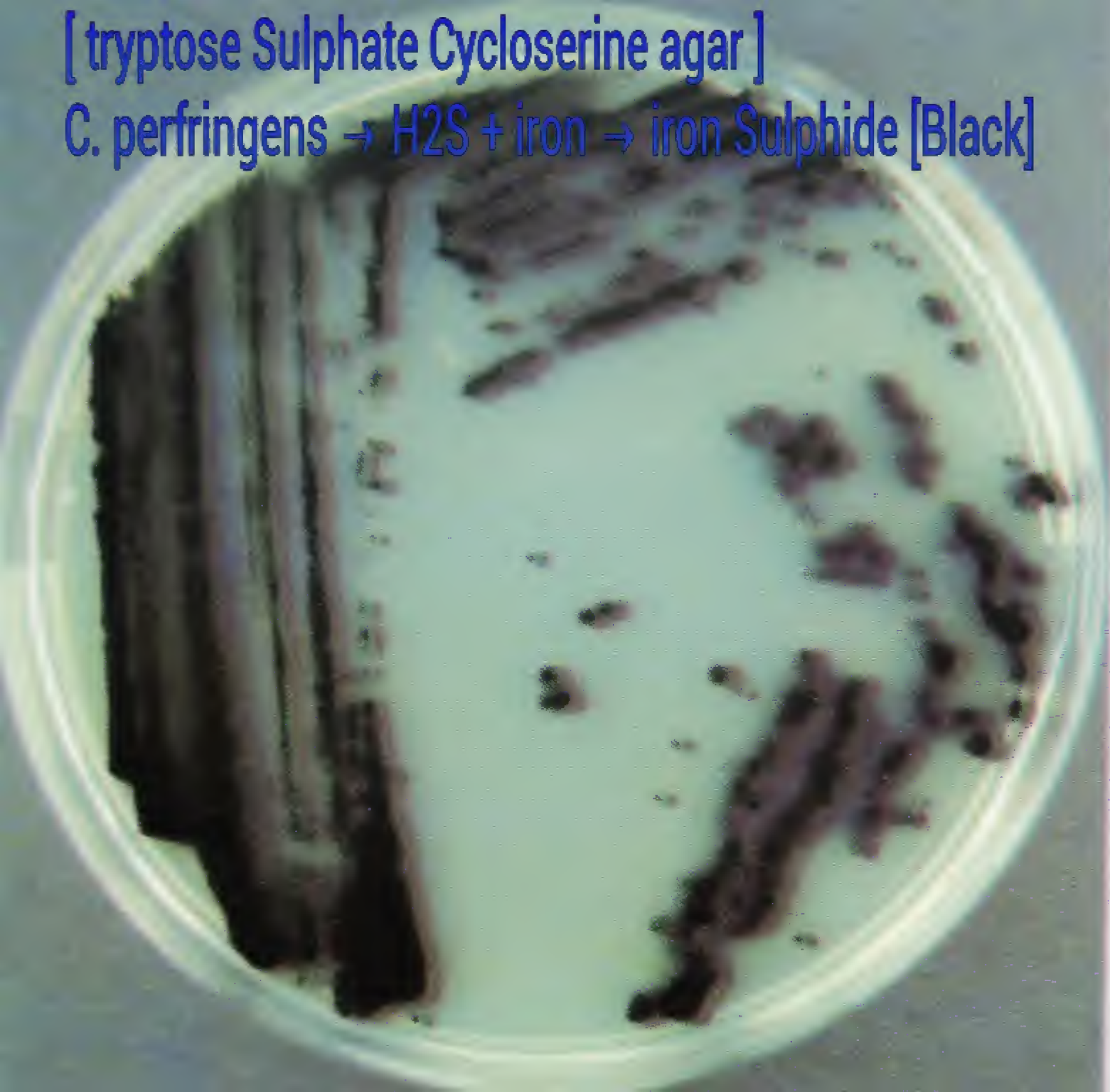
outer → partial haemolysis due to  $\alpha$ - Toxin release





[ tryptose Sulphate Cycloserine agar ]

*C. perfringens* →  $H_2S$  + iron → iron Sulphide [Black]



[ tryptose Sulphate Cycloserine agar ]

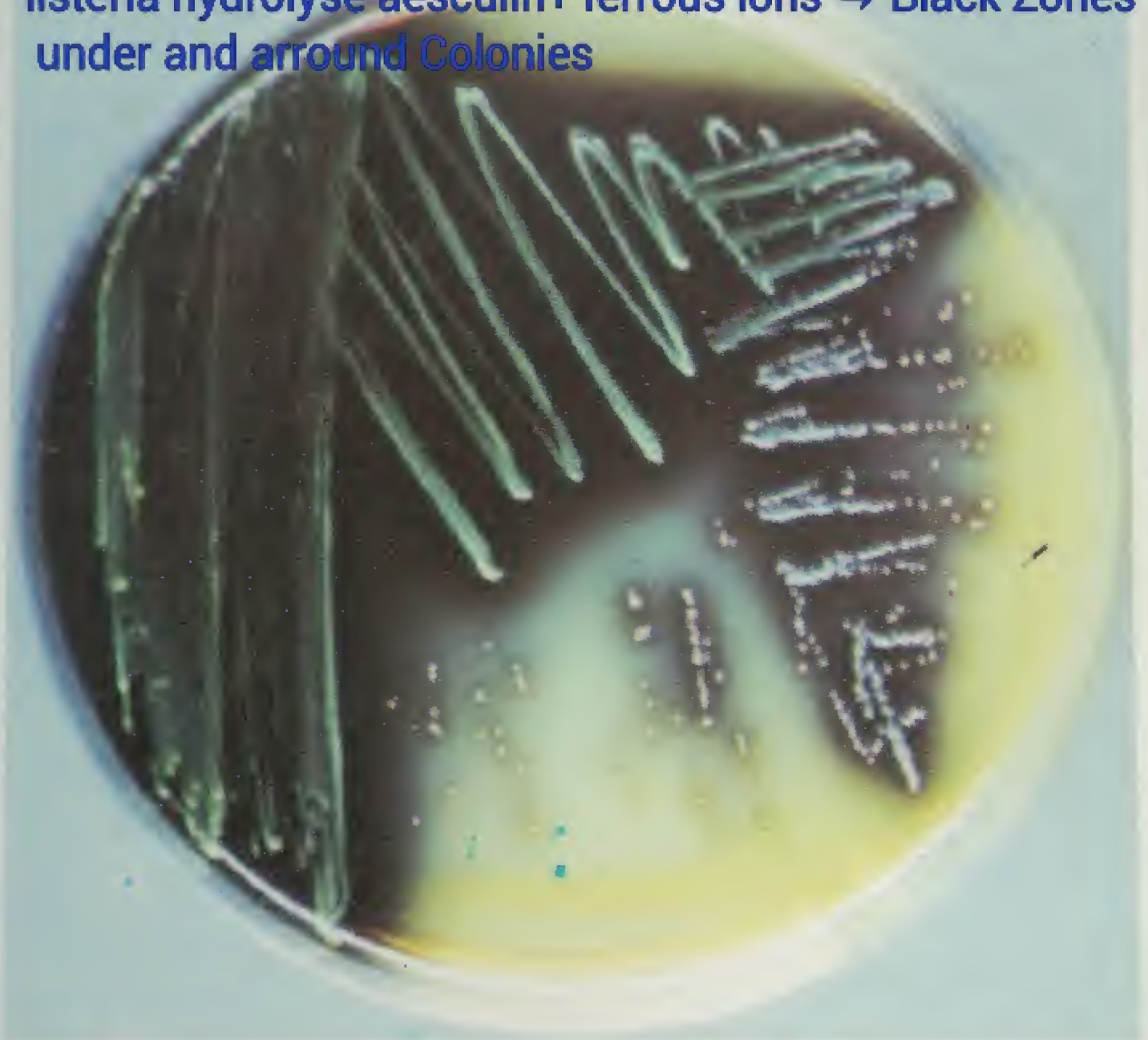
C. Sporogens → NO H<sub>2</sub>S → No Black Colour





[ Oxford agar for listeria SPP ]

listeria hydrolyse aesculin+ ferrous ions → Black Zones  
under and around Colonies



## [ Palcam agar for listeria SPP ]

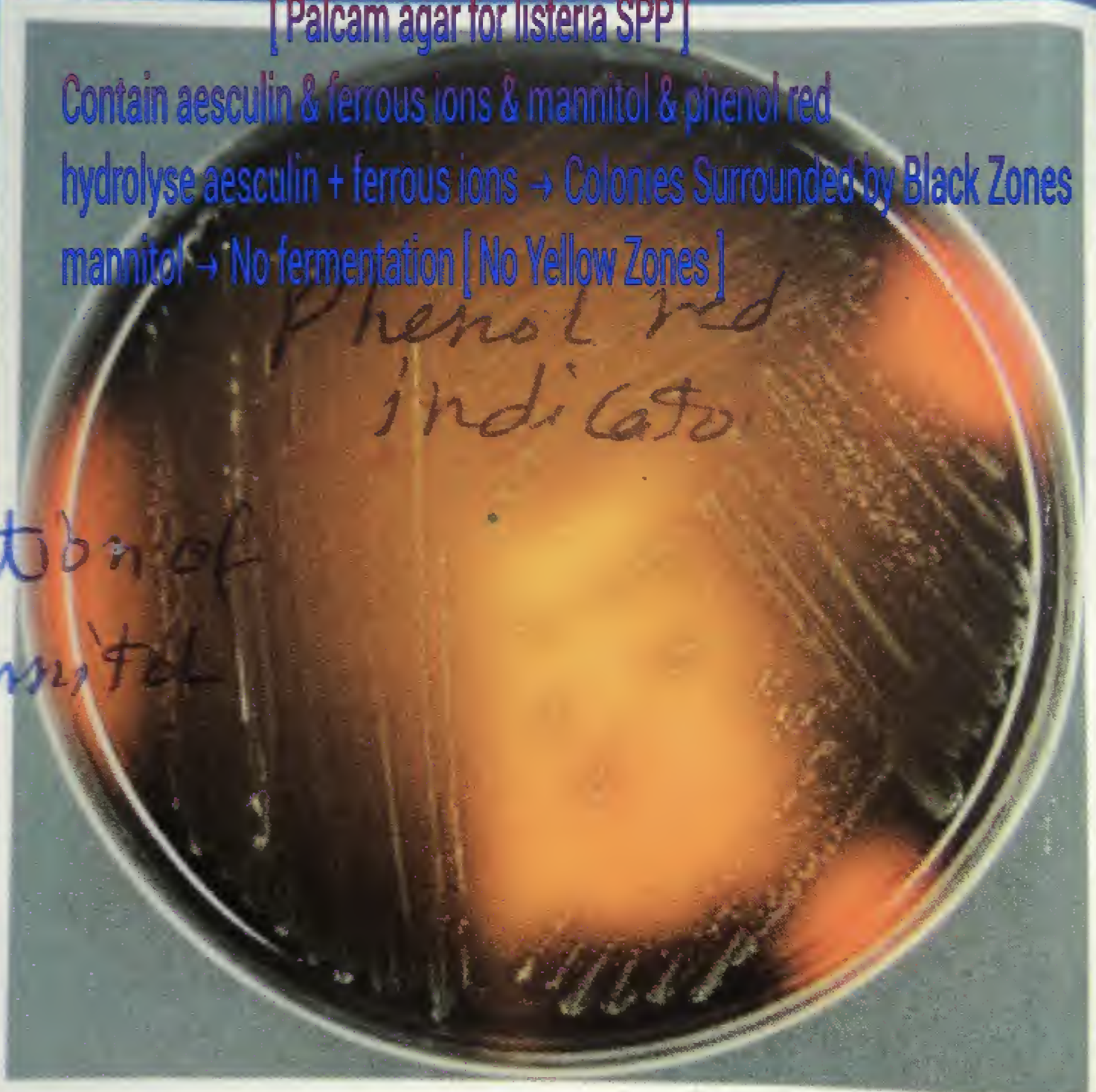
Contain aesculin & ferrous ions & mannitol & phenol red

hydrolyse aesculin + ferrous ions → Colonies Surrounded by Black Zones

mannitol → No fermentation [ No Yellow Zones ]

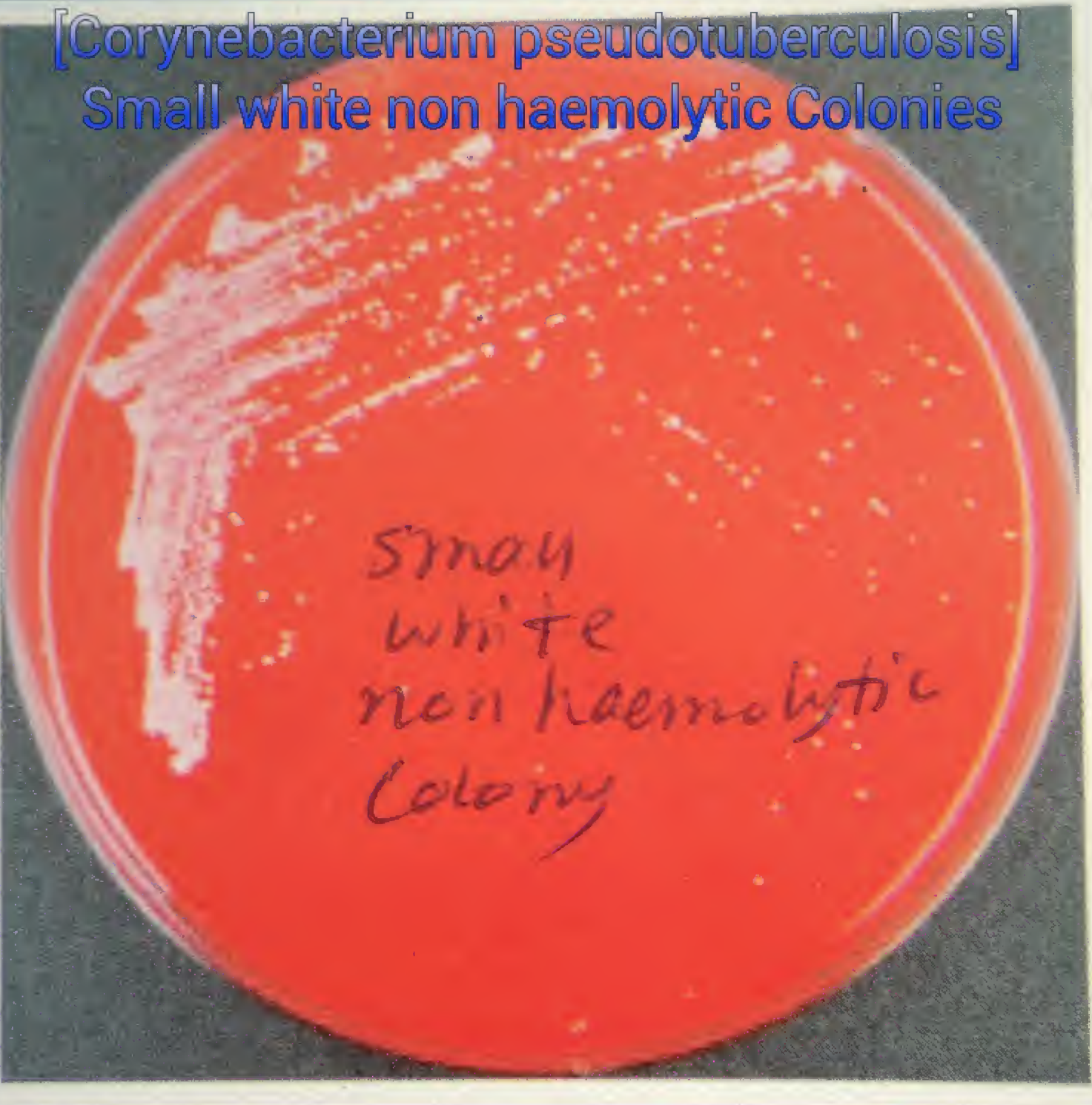
Phenol red  
indicator

hydrolysis of  
mannitol





[*Corynebacterium pseudotuberculosis*]  
Small white non haemolytic Colonies

A petri dish containing a red agar medium. On the left side, there is a streaked inoculation showing numerous small, white, non-haemolytic colonies. The rest of the dish is covered with a dense distribution of these same small white colonies.

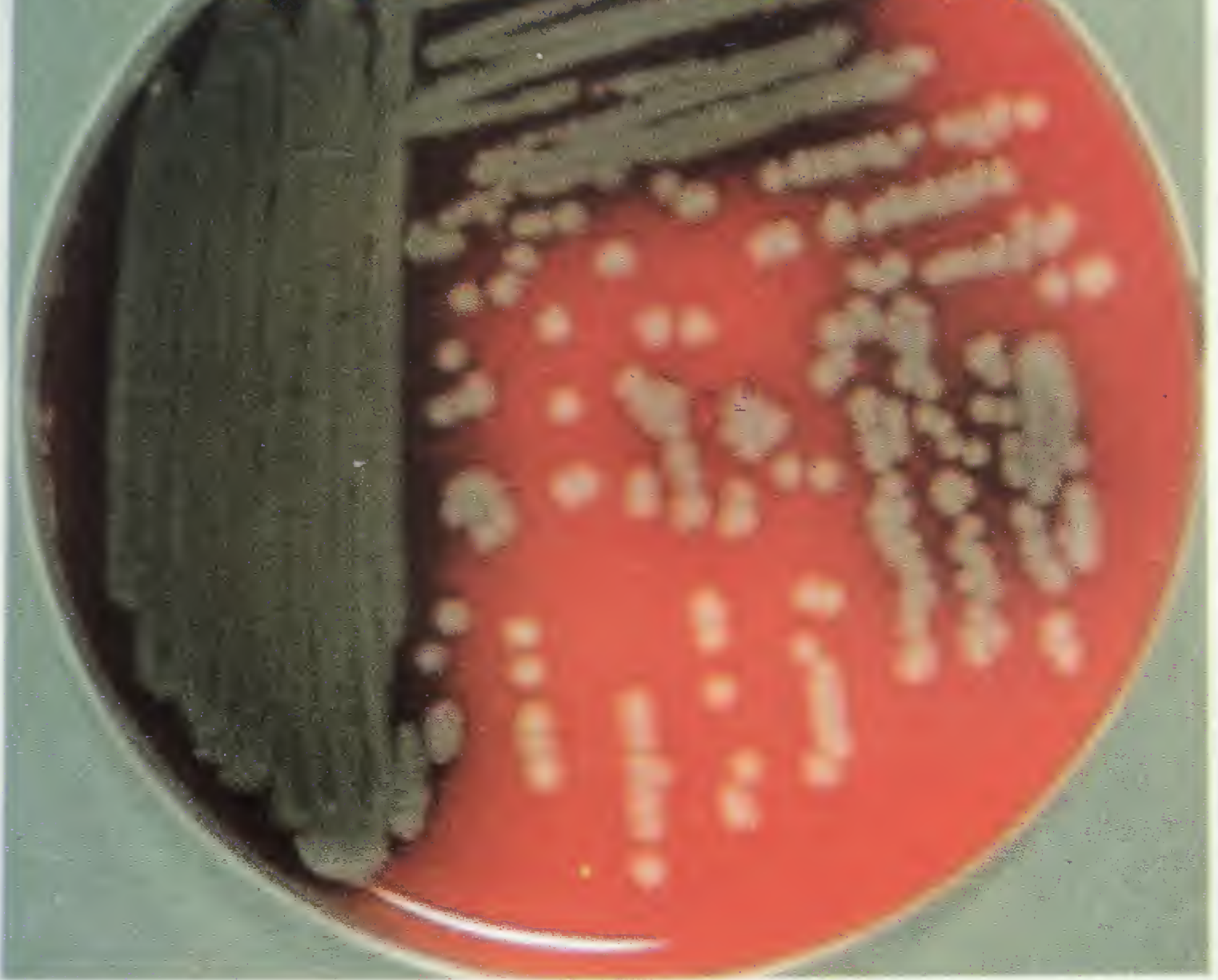
Small  
white  
non haemolytic  
Colonies

[ *Mycobacterium tuberculosis* Colonies  
On Lowenstein Jensen medium ]  
Dry , luxuriant , heavy growth [ eugenic ]  
yellowish Colony with rough Surface  
due to Cord factor

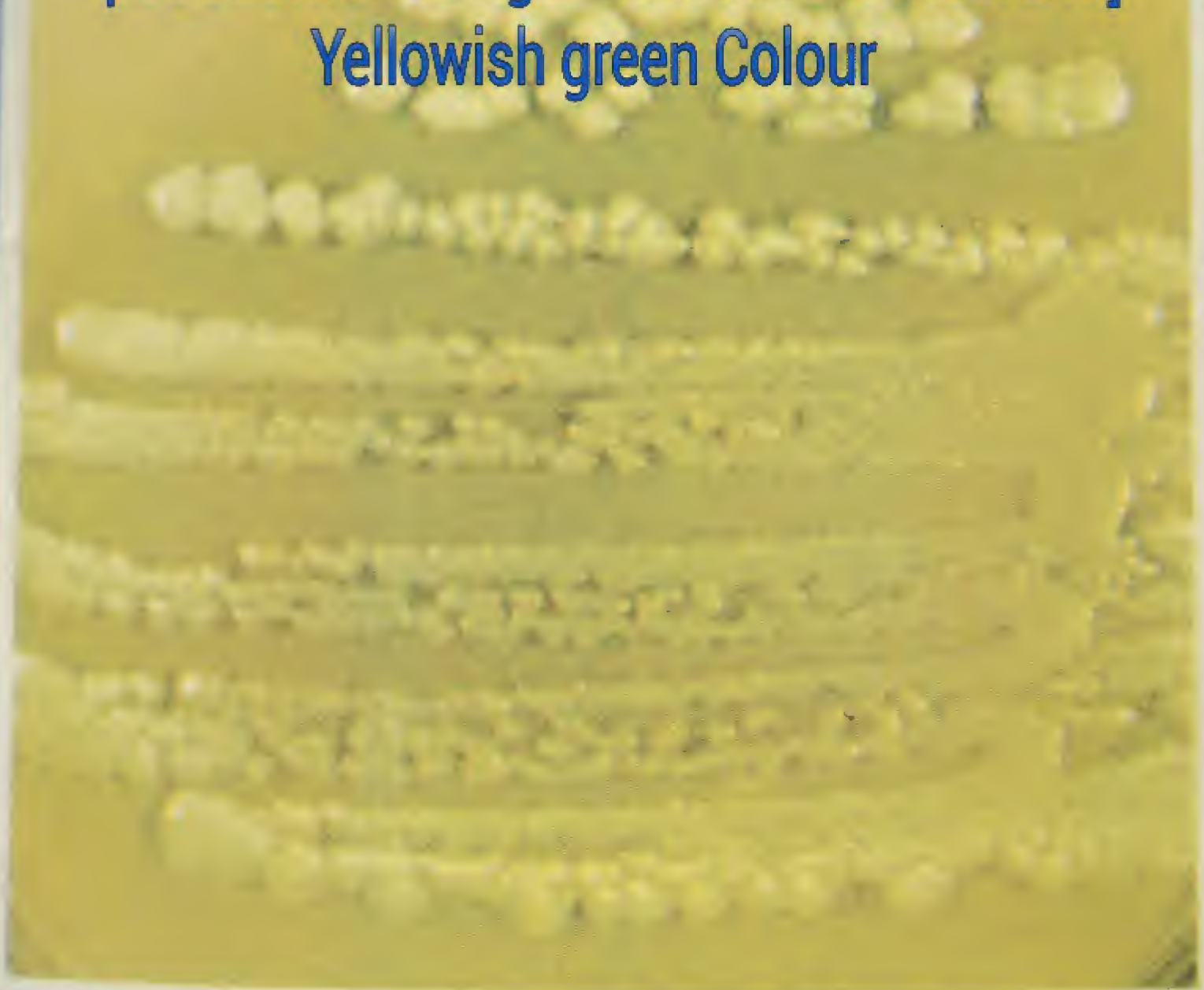





[ *Pseudomonas aeruginosa* on Blood agar ]  
large spreader haemolytic Colony



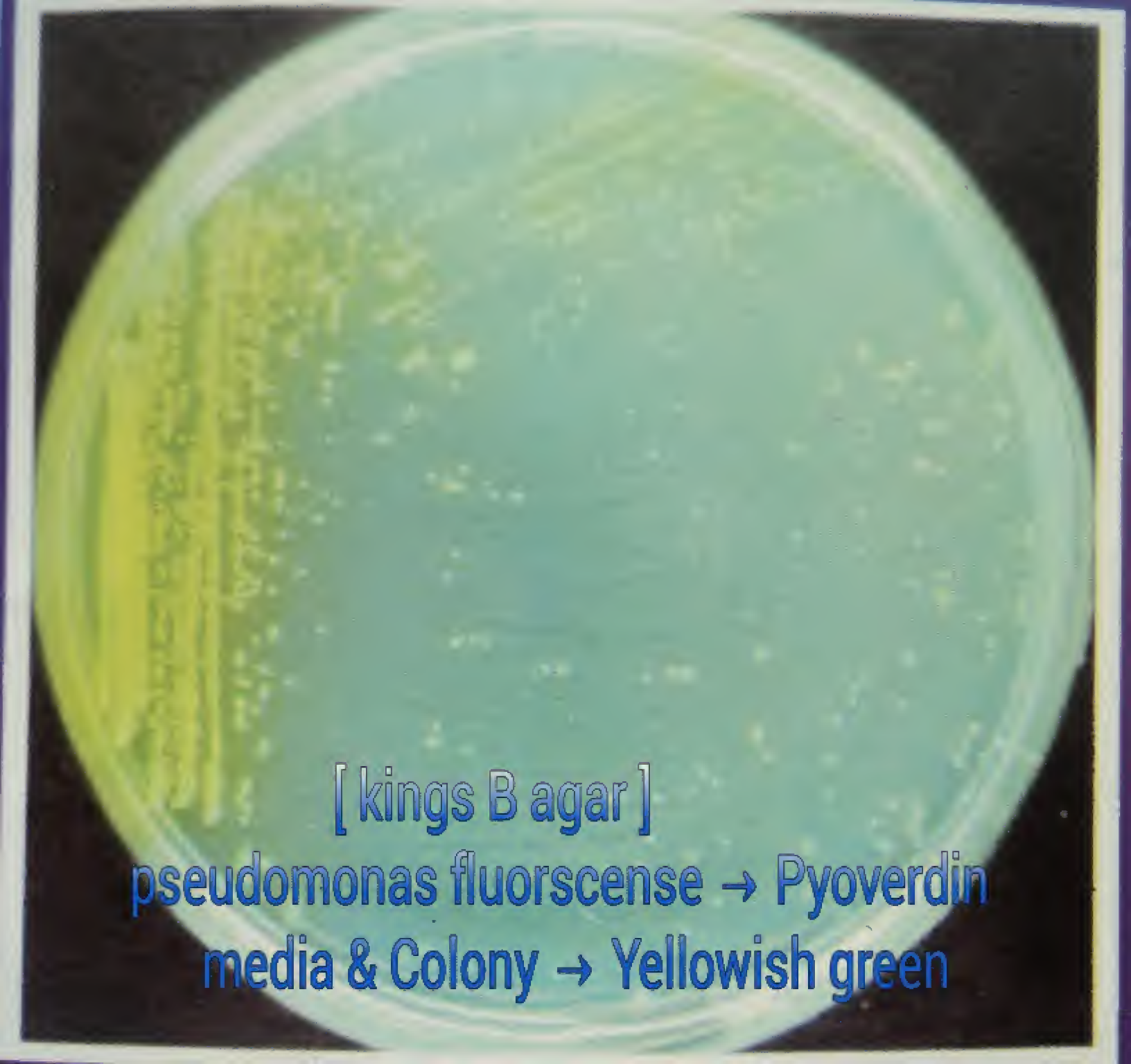
[ *Pseudomonas aeruginosa* on  
*Pseudomonas* agar fluorescent medium ]  
Yellowish green Colour







[ *Pseudomonas aeruginosa* on  
pseudomonas agar pyocyanin medium ]  
greenish blue Colour

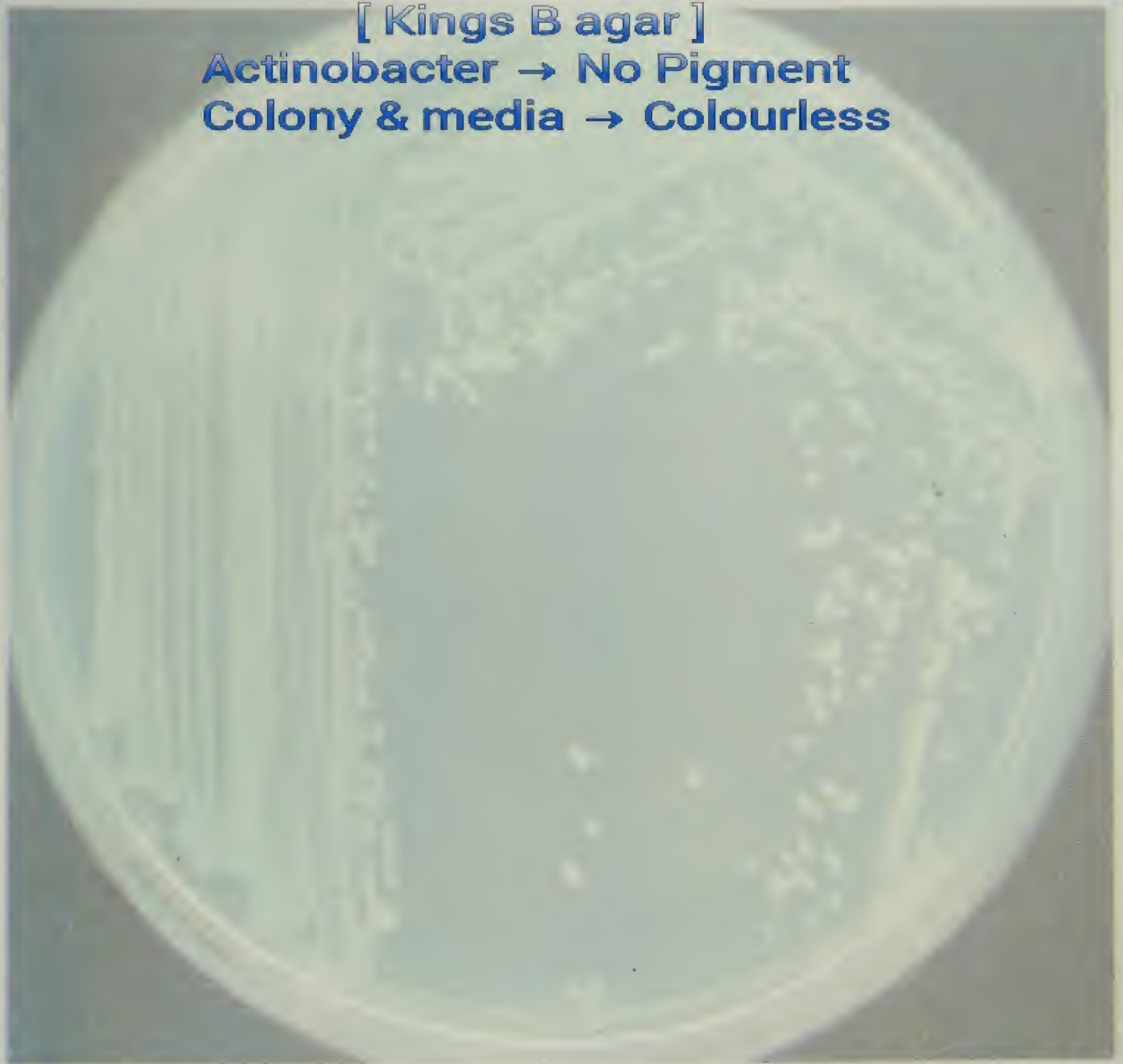
A photograph of a petri dish containing a bacterial culture on King's B agar. The agar is a light blue color. On the left side of the dish, there is a dense, vertical streak of bacterial growth that has turned a yellowish-green color. The rest of the agar surface appears relatively clear with some minor, scattered small colonies.

[ kings B agar ]

*pseudomonas fluorescens* → Pyoverdine  
media & Colony → Yellowish green



[ Kings B agar ]  
Actinobacter → No Pigment  
Colony & media → Colourless



[ Brucella ]

non haemolytic Small Colony on Blood agar

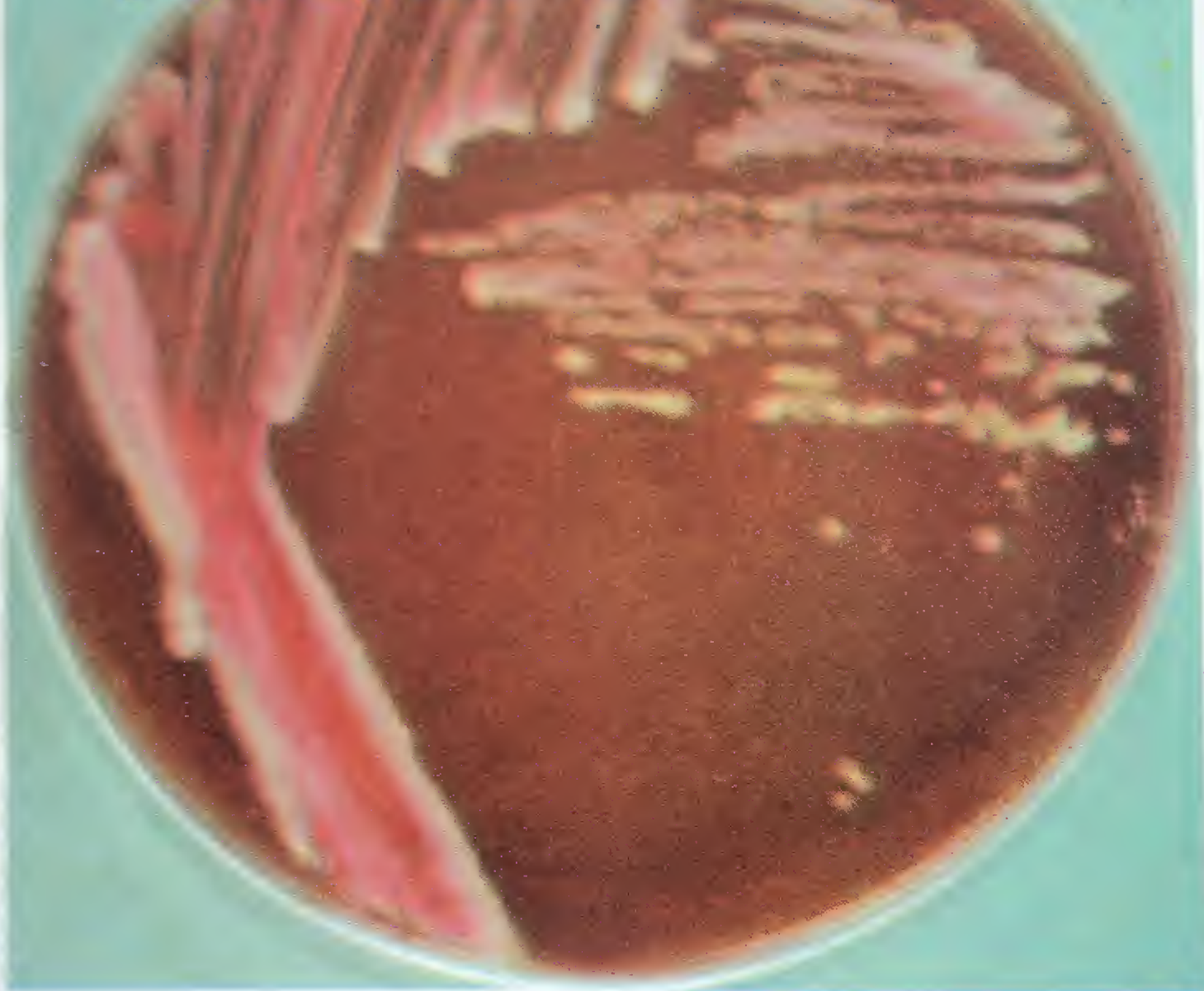




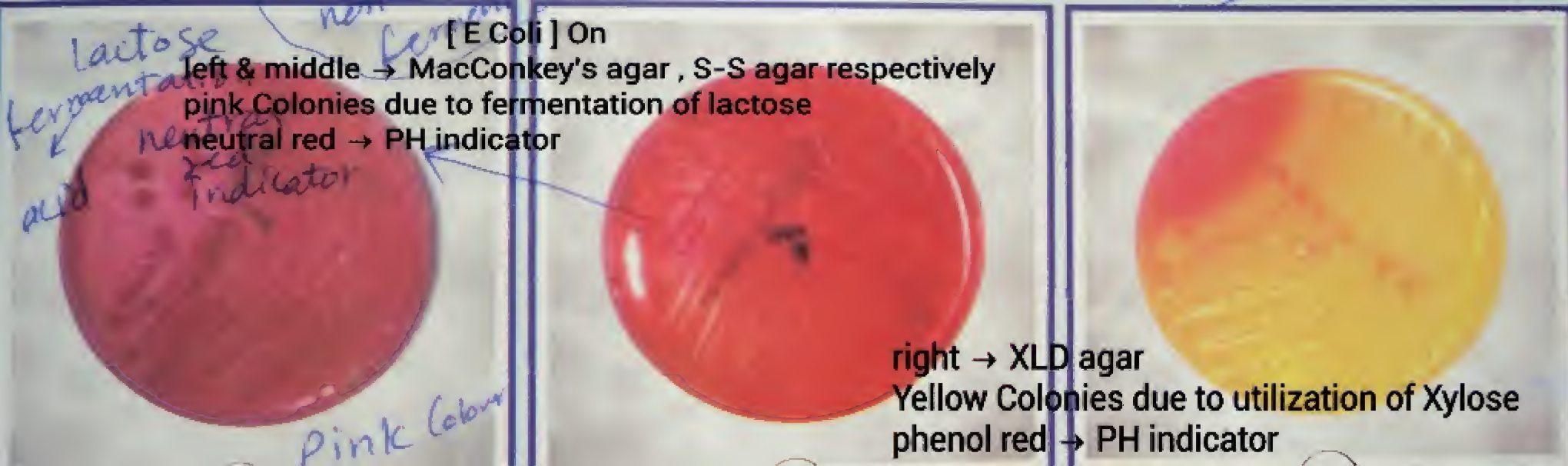
**[Bordetella bronchiseptica ]**  
**Non haemolytic Small Colony on Blood agar**



[ *Campylobacter jejuni* ]  
grayish spreader non haemolytic Colony







[ Klebsiella ] On

left & middle → MacConkey's agar, S-S agar respectively

Pink Colonies due to fermentation of lactose

neutral red → PH indicator



1

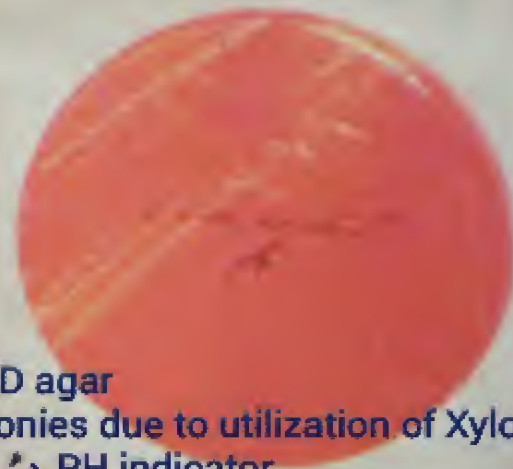


2

right → XLD agar

Yellow Colonies due to utilization of Xylose

phenol red → PH indicator



3

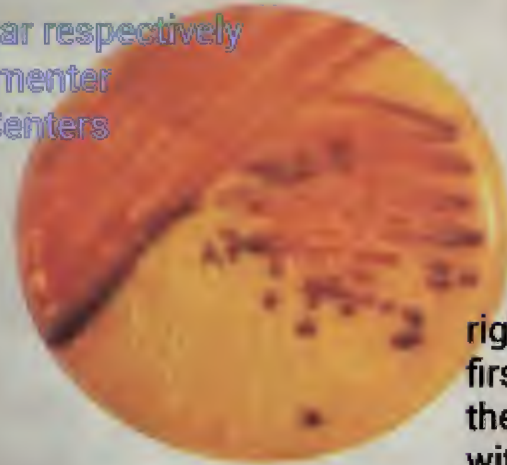


[ Salmonella ] On

left & middle → MacConkey's agar, S-S agar respectively

Colourless Colonies due to non lactose fermenter

Middle →  $H_2S$  production + iron → Black Centers

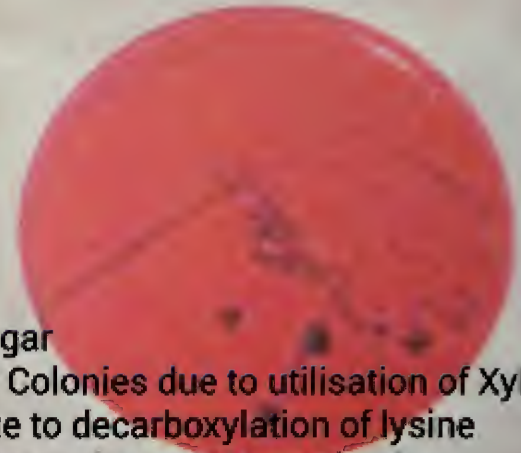


right → XLD agar

first → Yellow Colonies due to utilisation of Xylose

then → red due to decarboxylation of lysine

with black Centers due to  $H_2S$  production



[E Coli on Eosin Methylene Blue ] EMB agar  
Sucrose + Lactose → fermentation and formation of  
Methylene Blue eosinate Complex [ metallic Sheen ]

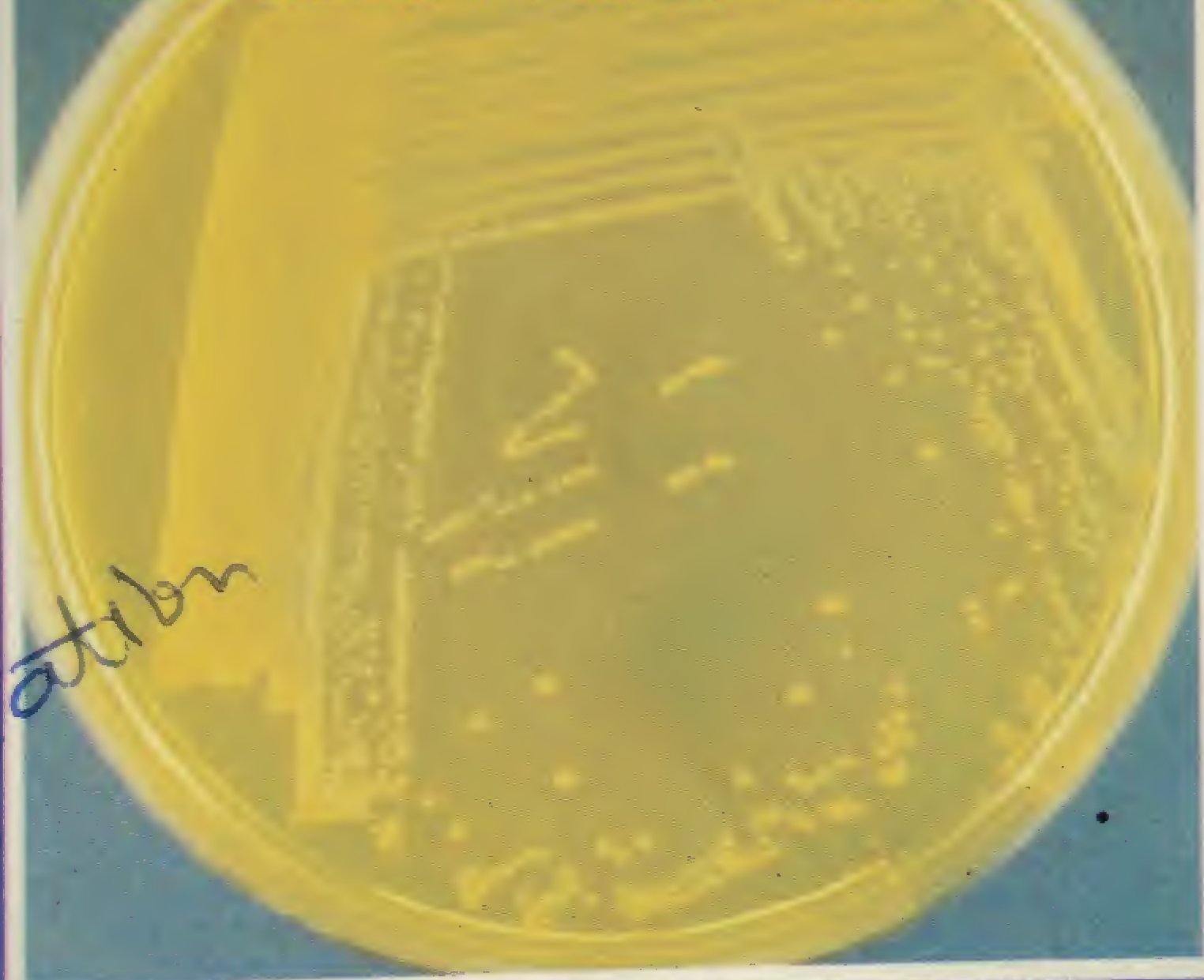




**[ Klebsiella on Eosin Methylene Blue ] EMB agar**  
**Colourless or light Purple Colonies**  
**no Sucrose and lactose**

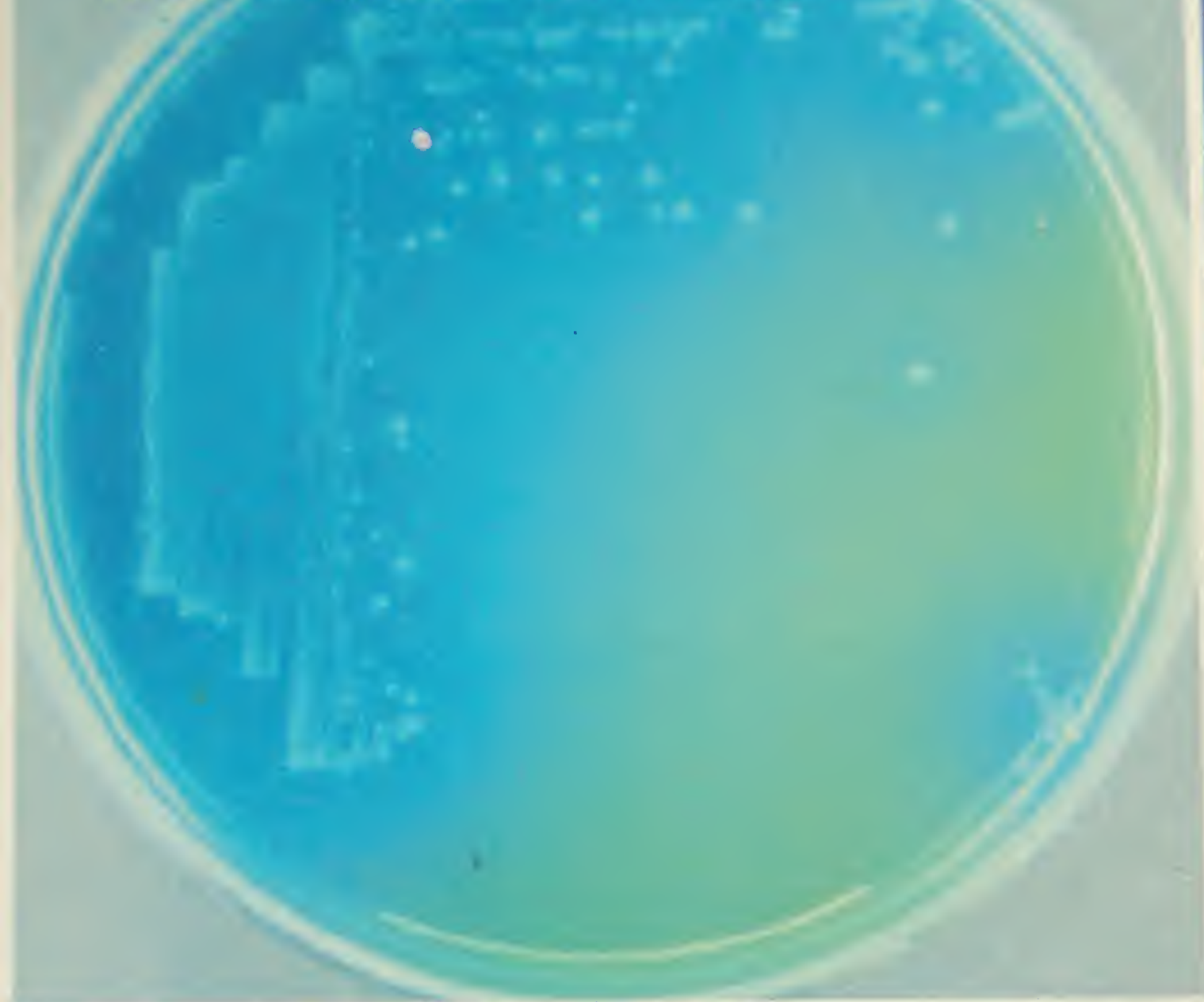


[ E Coli on Bromothymol Blue lactose Sucrose ] agar  
ferment lactose and / or Sucrose → Yellow Colonies



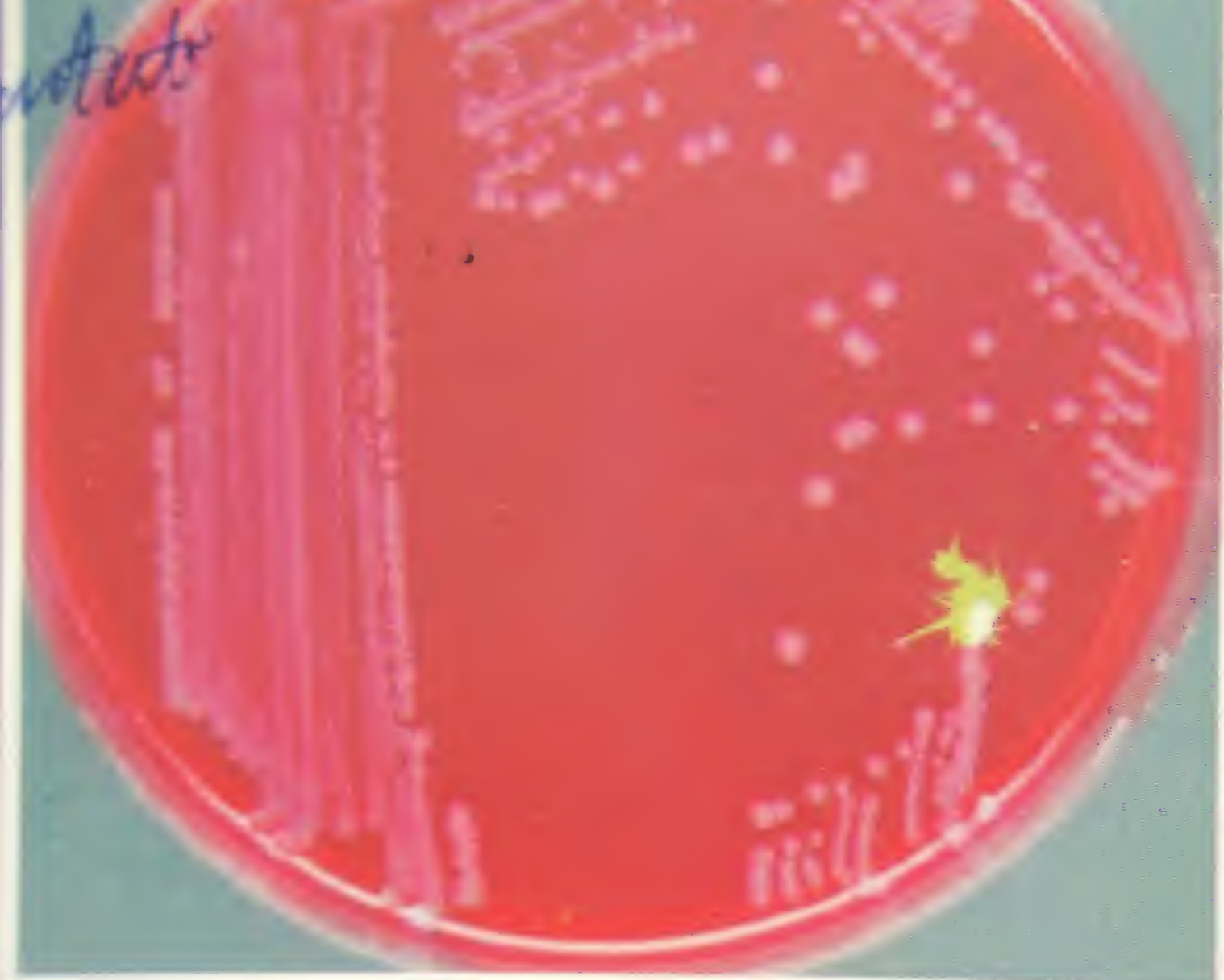


[ Salmonella on Bromothymol Blue lactose Sucrose ] agar  
not ferment lactose or Sucrose → Blue Colonies



BPLS [ Brilliant-green phenol-red lactose Sucrose ] agar

Salmonella → red Colonies due to no fermentation of Lactose or sucrose





BPLS [ Brilliant-green phenol-red lactose Sucrose ] agar  
fermentation of lactose and / or Sucrose → Acid  
→ low PH → Yellow Colonies

E Coli

Tat 16 m



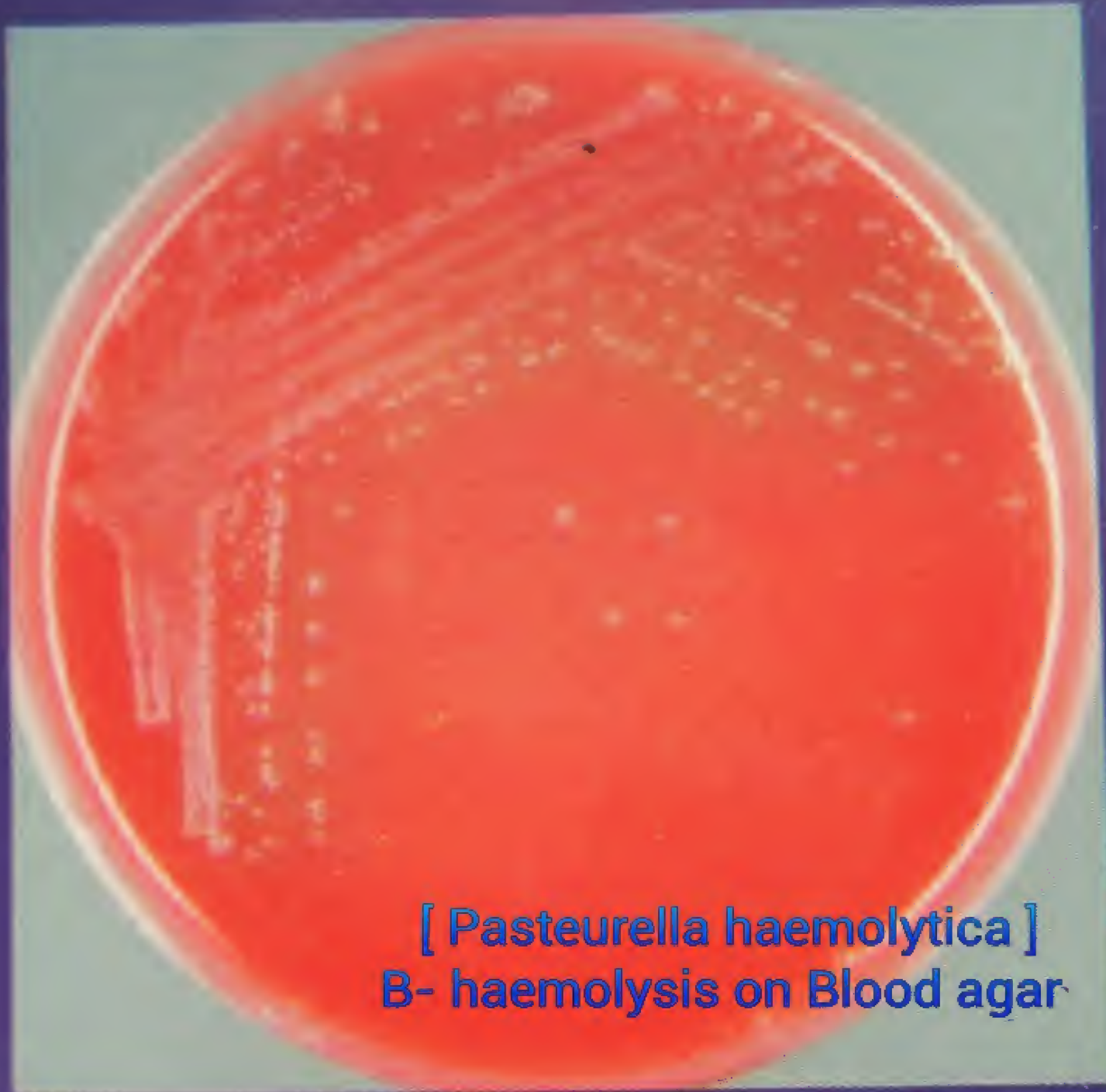
[ *Proteus mirabilis* ]  
Swarming on Blood agar due to  
active Motile Microorganism







[ *Pasteurella Multocida* ]  
non haemolytic white Colony  
on Blood agar



[ *Pasteurella haemolytica* ]  
B- haemolysis on Blood agar



# [ Mycoplasma Micro Colonies ]

fried egg Micro Colony

Opaque Center with transparent peripheral Zone



# [ Mycoplasma Micro Colonies ]

fried egg Micro Colony

Opaque Center with transparent peripheral Zone

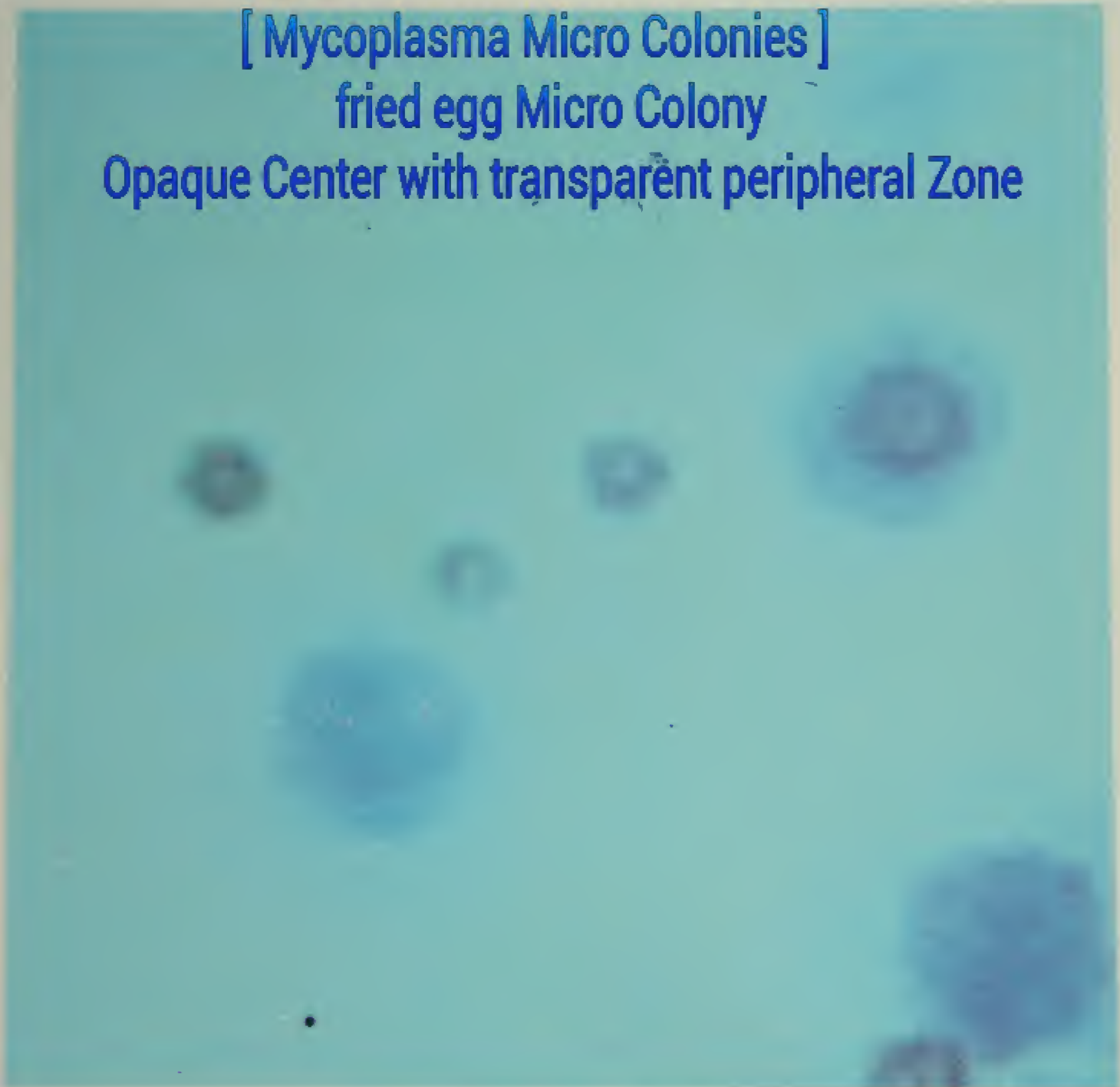




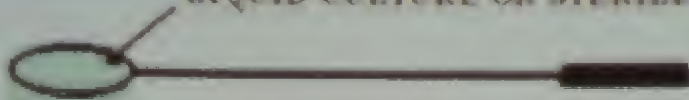
# [ Mycoplasma Micro Colonies ]

fried egg Micro Colony

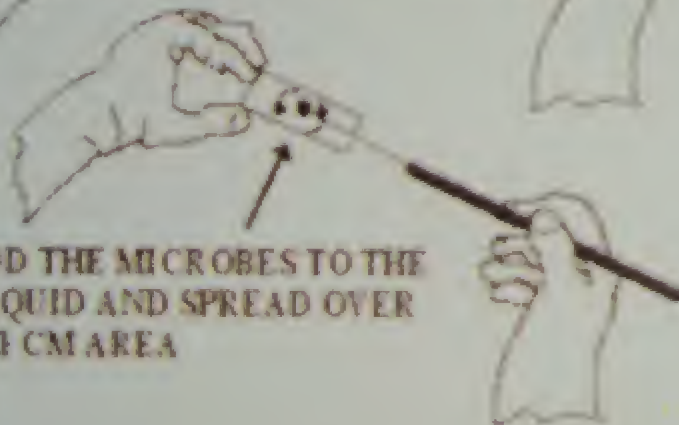
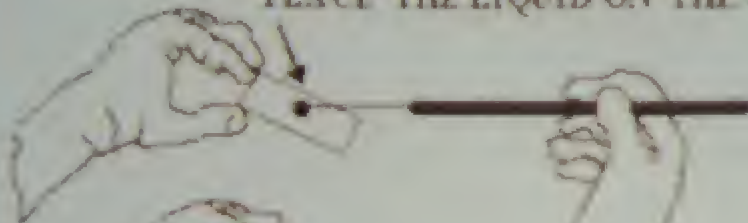
Opaque Center with transparent peripheral Zone



LIQUID CULTURE OR STERILE WATER



PLACE THE LIQUID ON THE SLIDE



ADD THE MICROBES TO THE  
LIQUID AND SPREAD OVER  
A 1 CM AREA

AIR DRY OR HEAT GENTLY. WHEN DRY  
BRIEFLY HEAT FIX THE CELLS TO THE  
SLIDE

## Preparation of bacterial film



**Gram Positive**



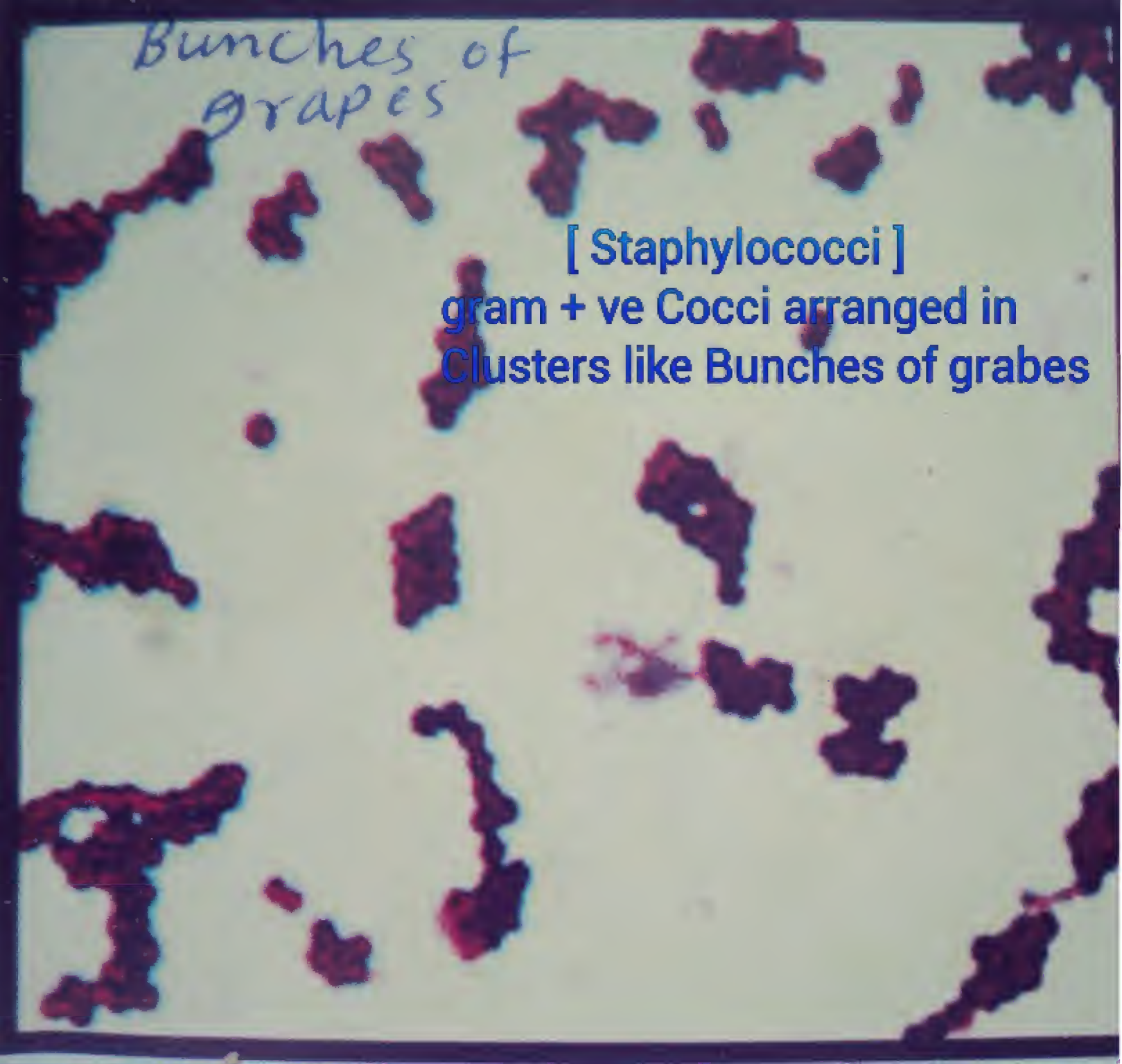
**Gram Negative**



**Gram's stain**

Bunches of  
grapes

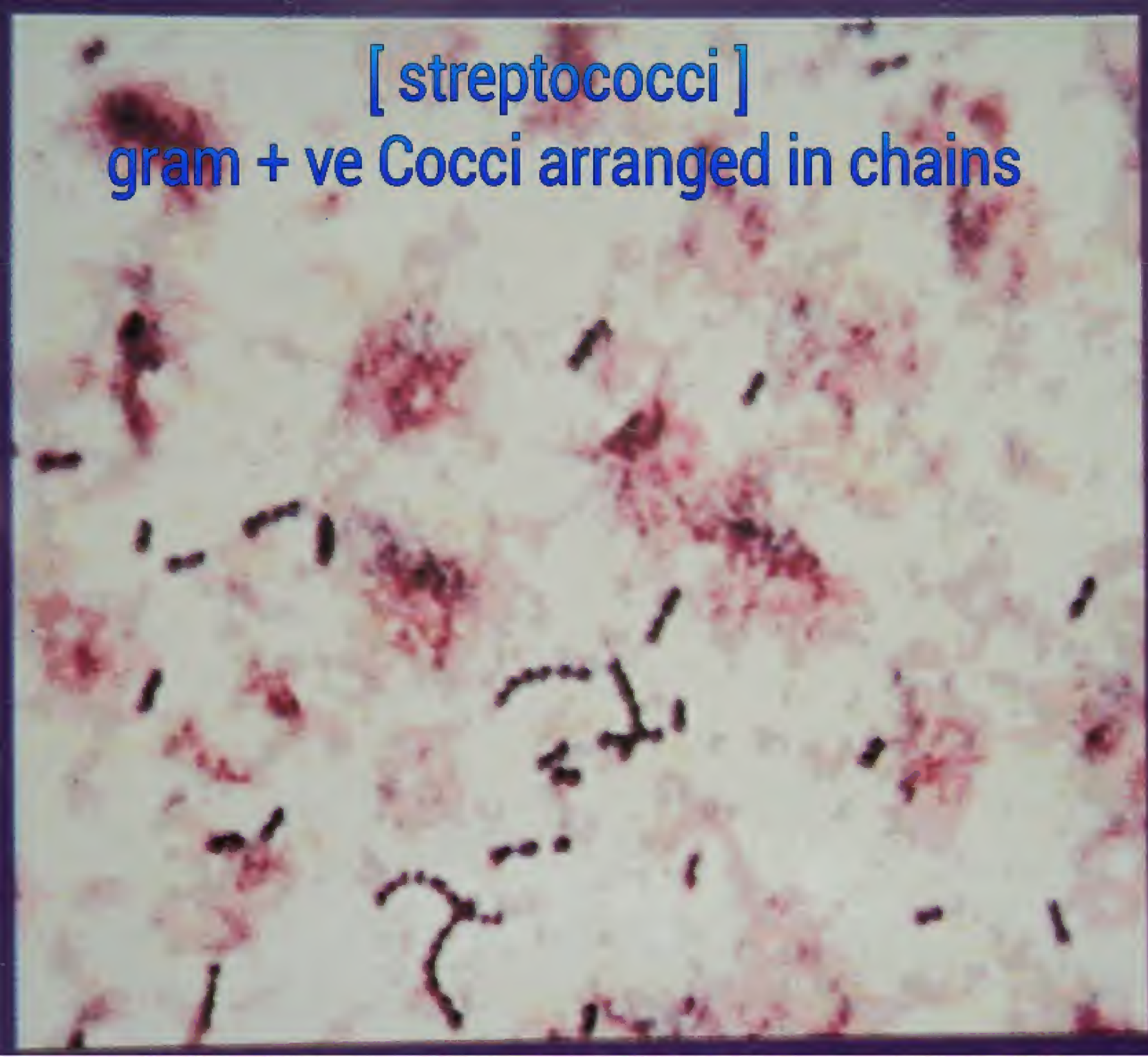
[ Staphylococci ]  
gram + ve Cocci arranged in  
Clusters like Bunches of grapes



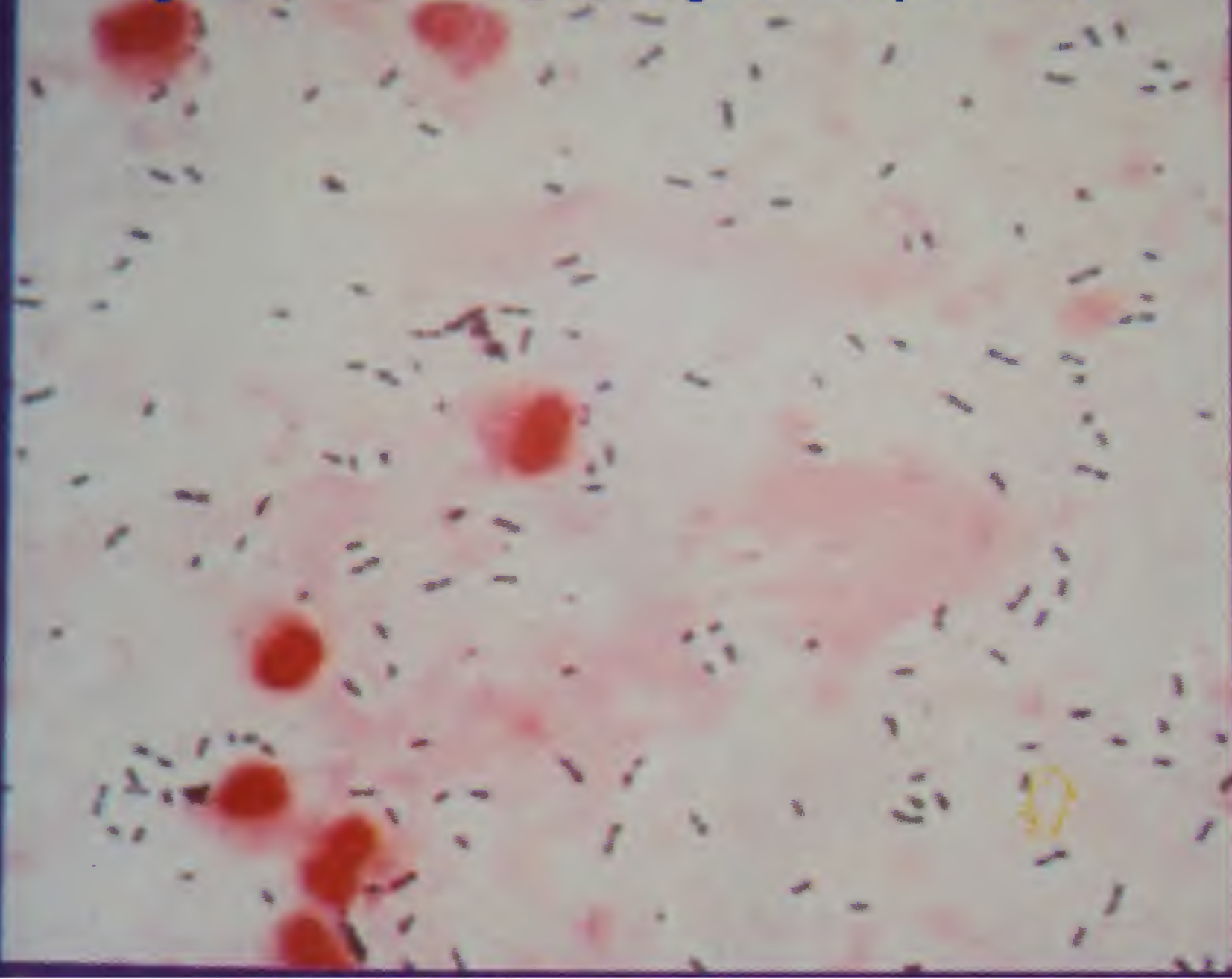


[ streptococci ]

gram + ve Cocci arranged in chains

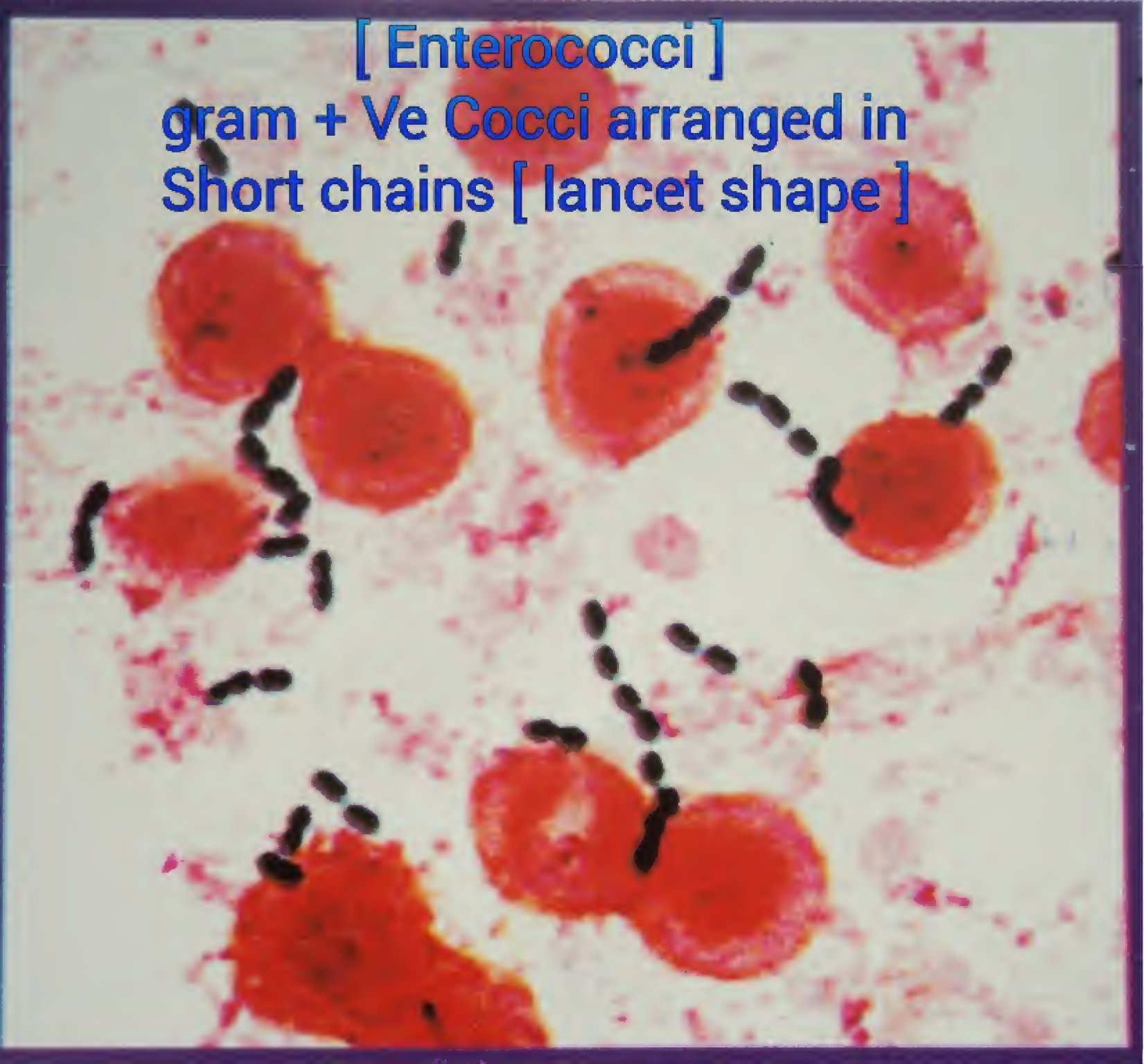


[ *Diplo Coccus pneuminae* ]  
gram + ve Cocci arranged in Diplo cocci





**[ Enterococci ]**  
**gram + Ve Cocci arranged in**  
**Short chains [ lancet shape ]**

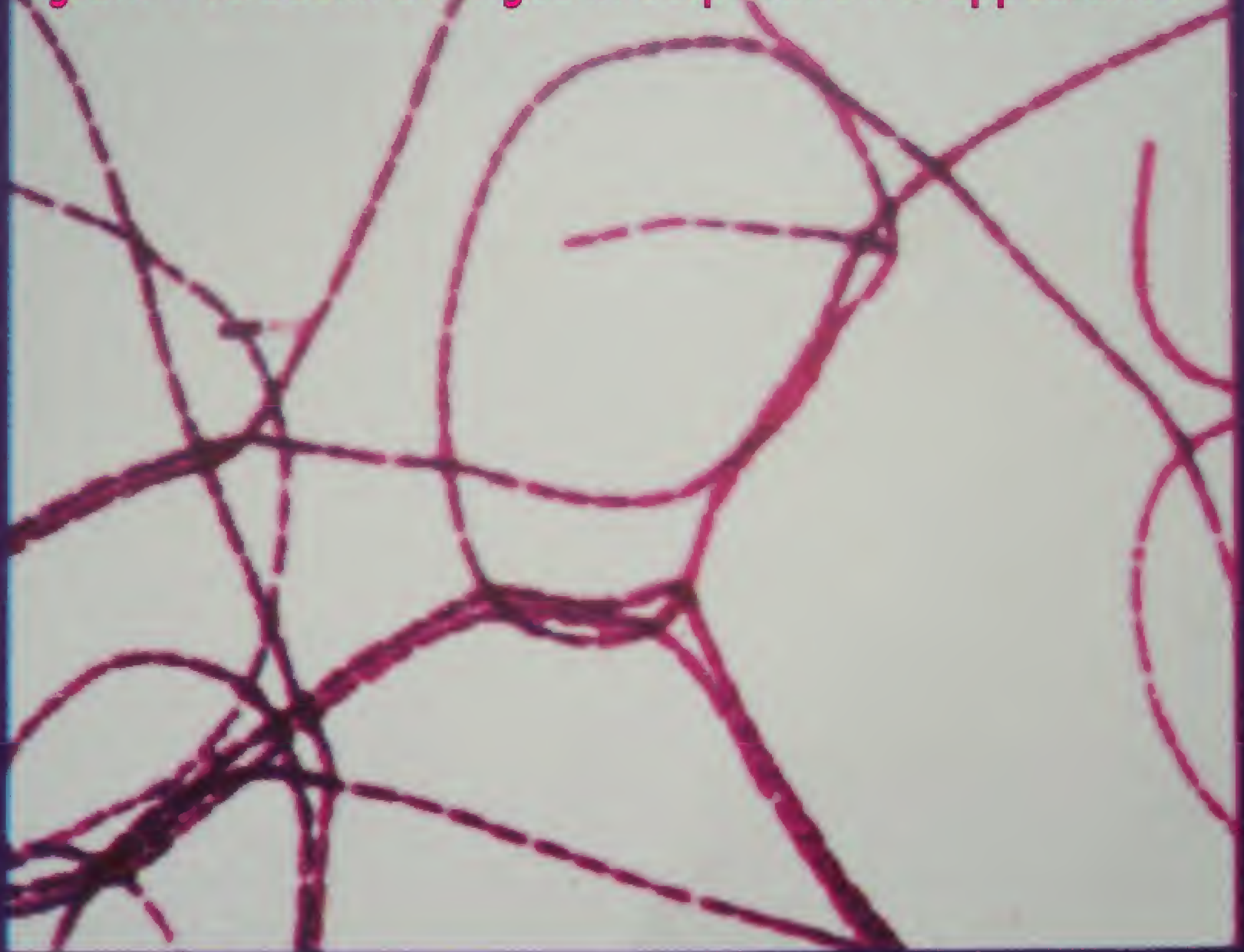




[ *B. anthracis* from Culture Containing oxalates]  
gram + ve Bacilli arranged in Chains with Central  
oval Spore



[ *B. anthracis* from Culture Containing Calcium Chloride ]  
gram + ve Bacilli arranged in Serpentine like appearance





# [ B. Cereus ] gram + ve Bacilli





**[ C. tetani ]**  
**gram + Ve Bacilli with**  
**drumstick like appearance**

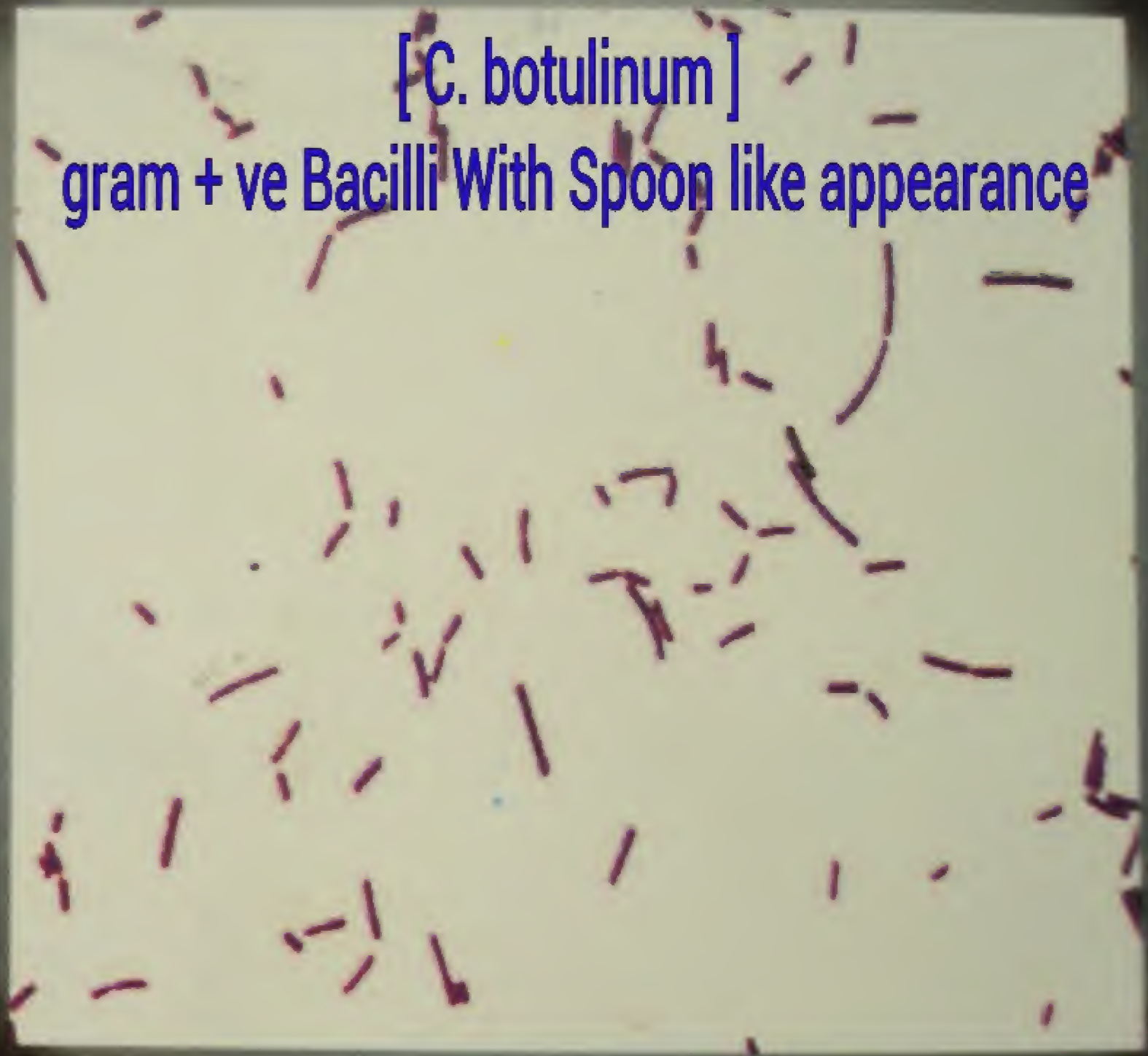


[ *C. botulinum* ]  
gram + ve Bacilli With Spoon like appearance



[ *C. botulinum* ]

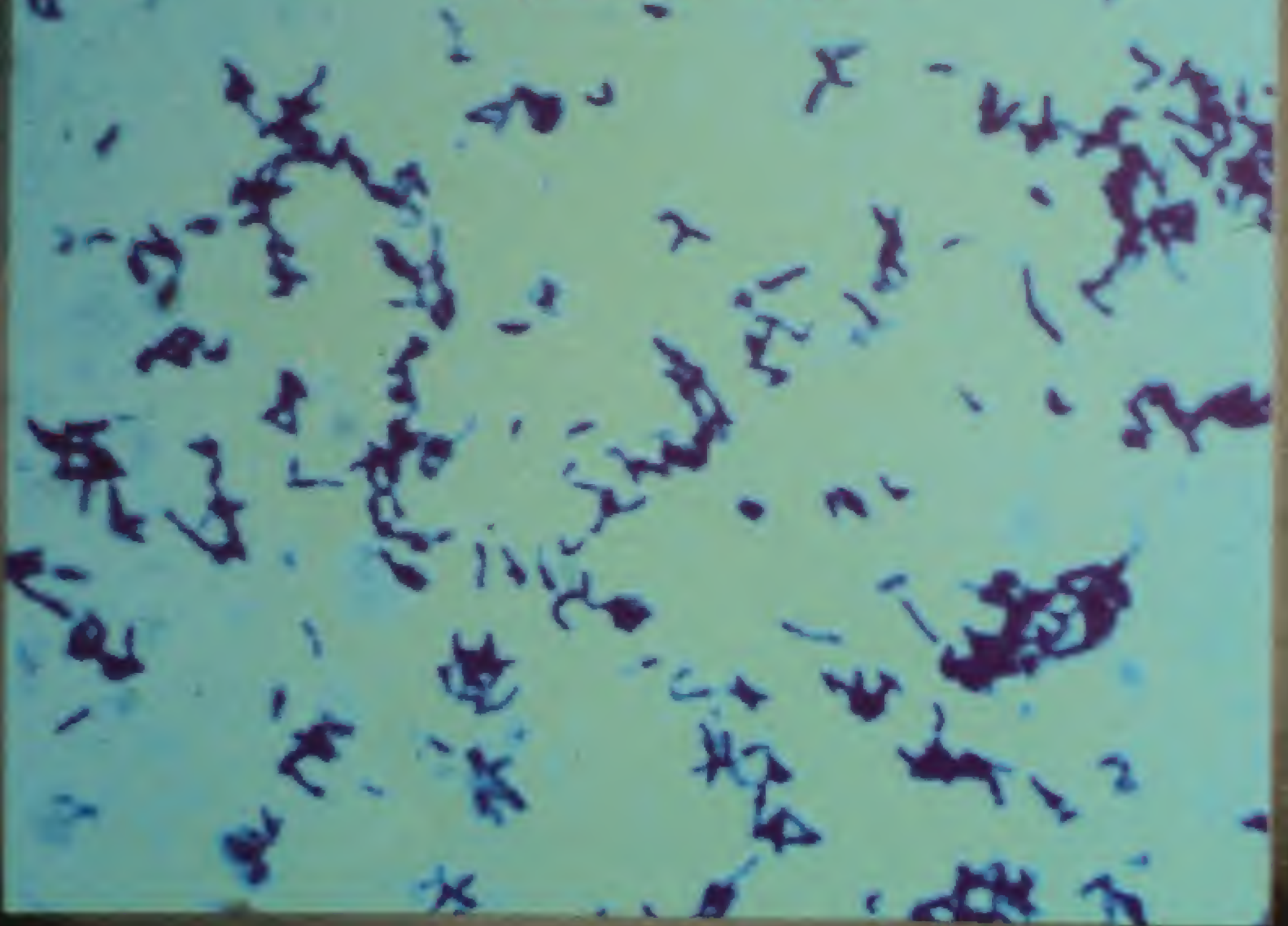
gram + ve Bacilli With Spoon like appearance





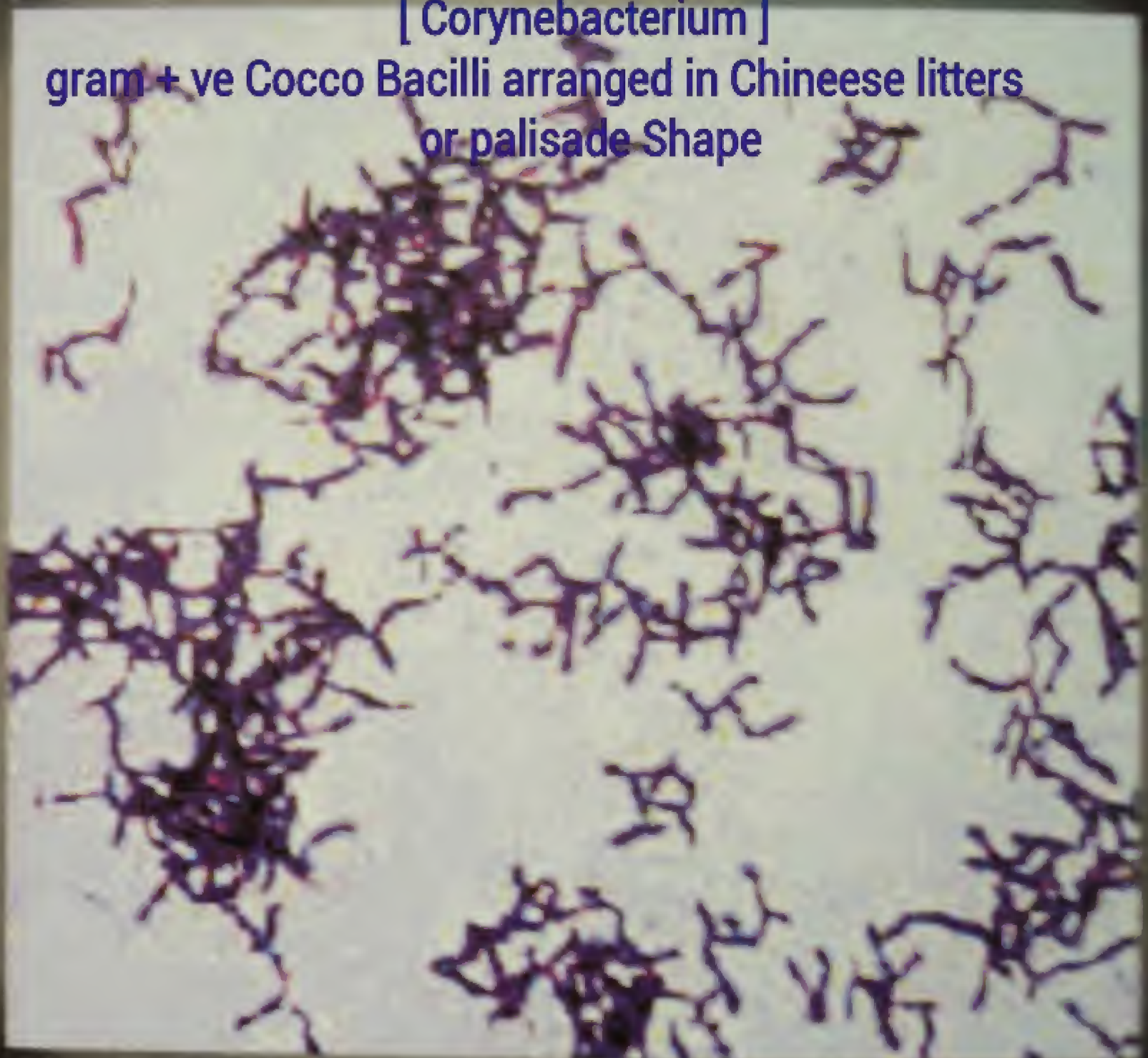
[ *Corynebacterium* ]

gram + ve Cocco Bacilli arranged in Chinese litters  
or palisade Shape



[ *Corynebacterium* ]

gram + ve Cocco Bacilli arranged in Chinese litters  
or palisade Shape



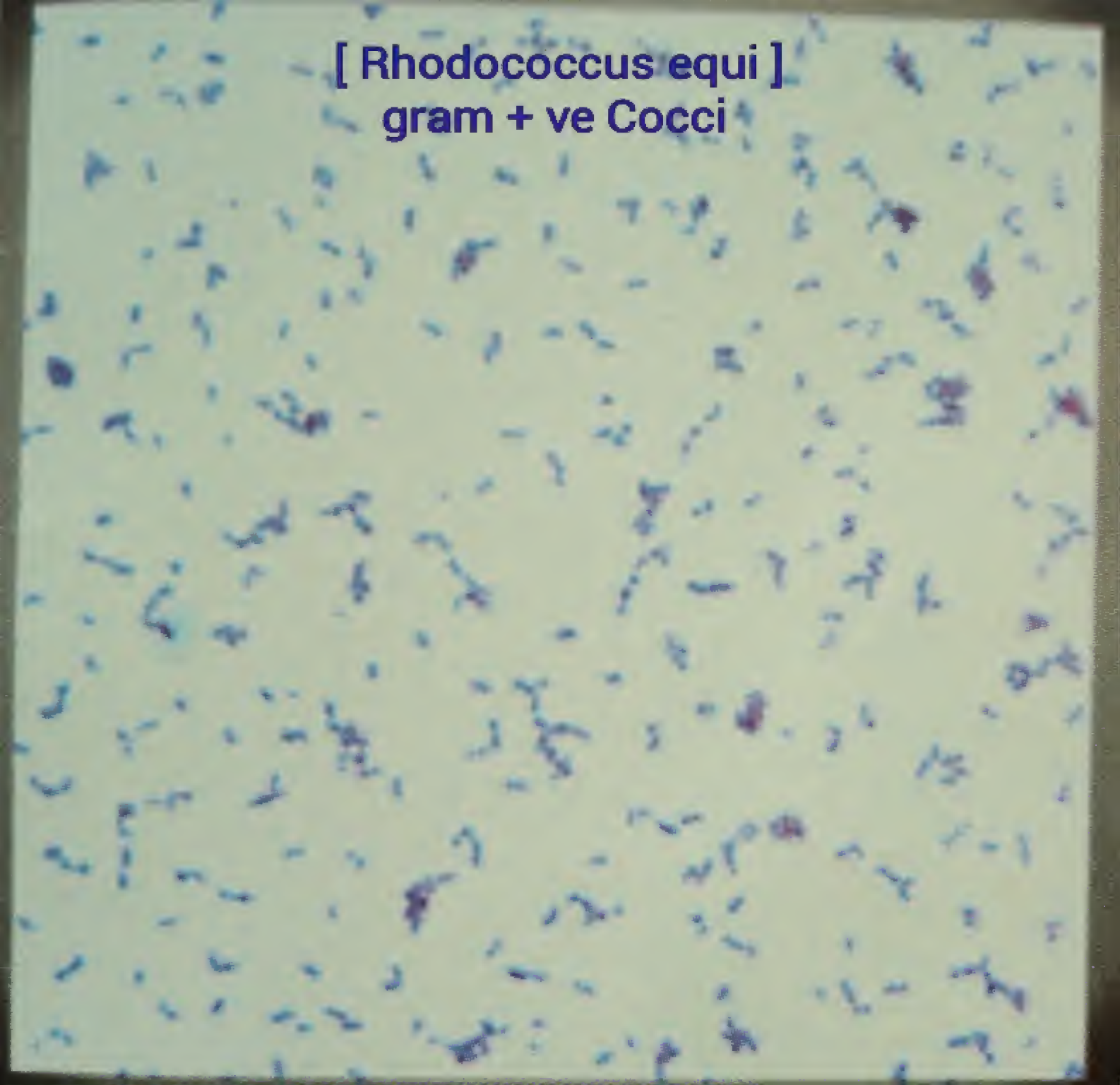


A microscopic image showing numerous small, pinkish-red, rod-shaped bacteria (cocci) scattered across a light-colored background. The bacteria are arranged in various patterns, including single cells, pairs, and small clusters. The overall appearance is that of a Gram-stained smear.

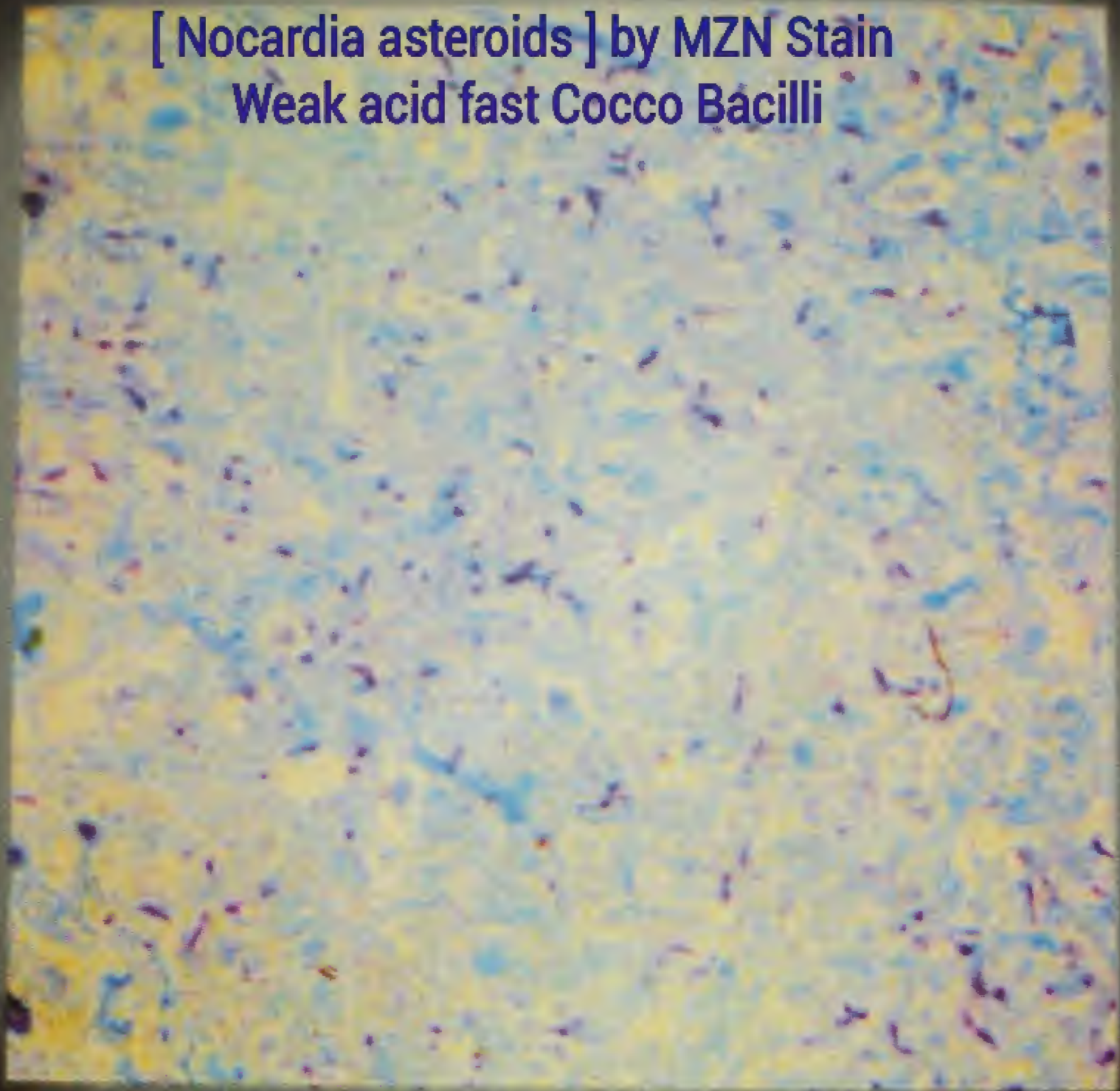
[ listeria ]  
gram + ve Cocco Bacilli



[ *Rhodococcus equi* ]  
gram + ve Cocci



[ *Nocardia asteroides* ] by MZN Stain  
Weak acid fast Cocco Bacilli

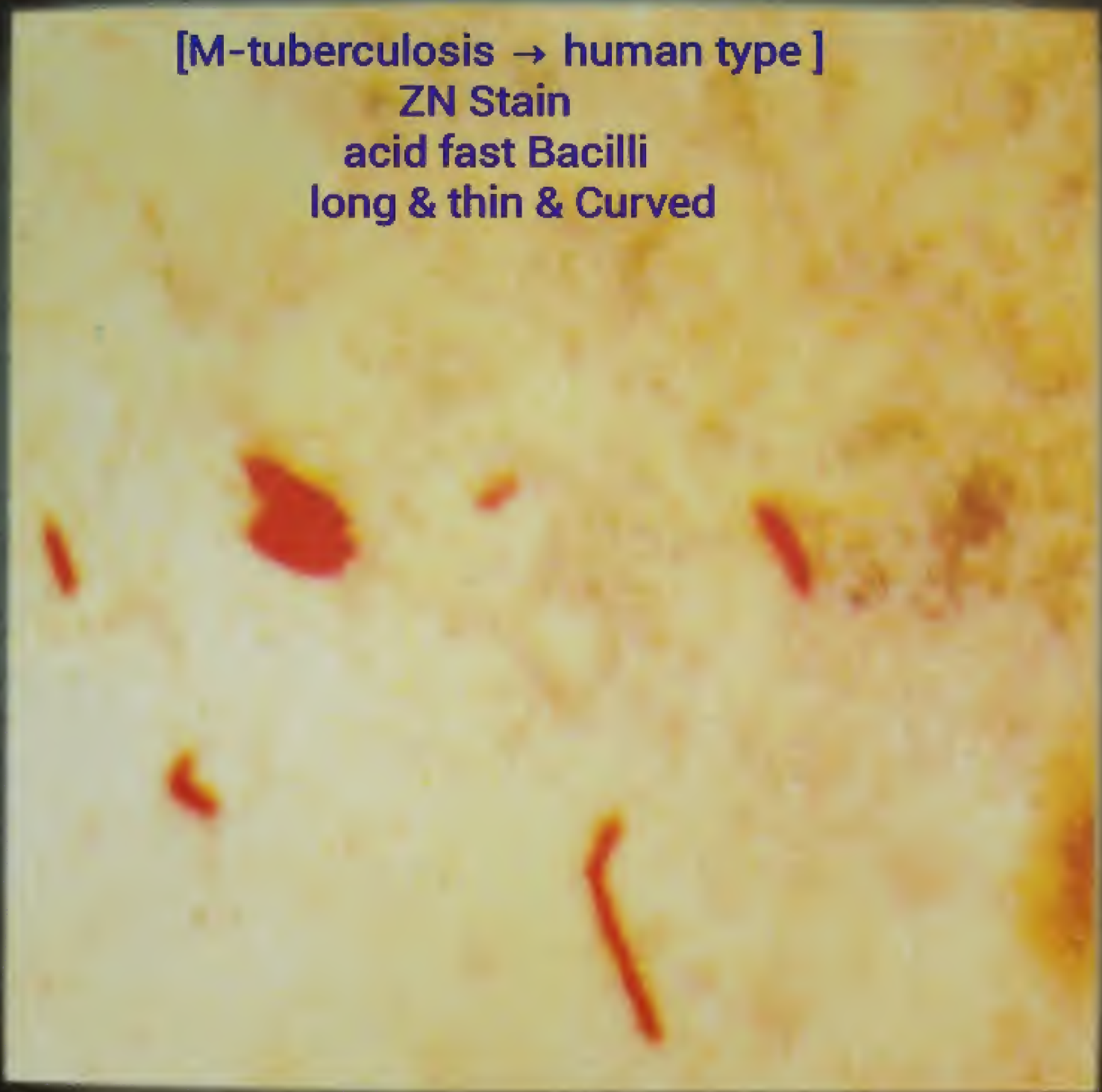


**[M-tuberculosis → human type ]**

**ZN Stain**

**acid fast Bacilli**

**long & thin & Curved**





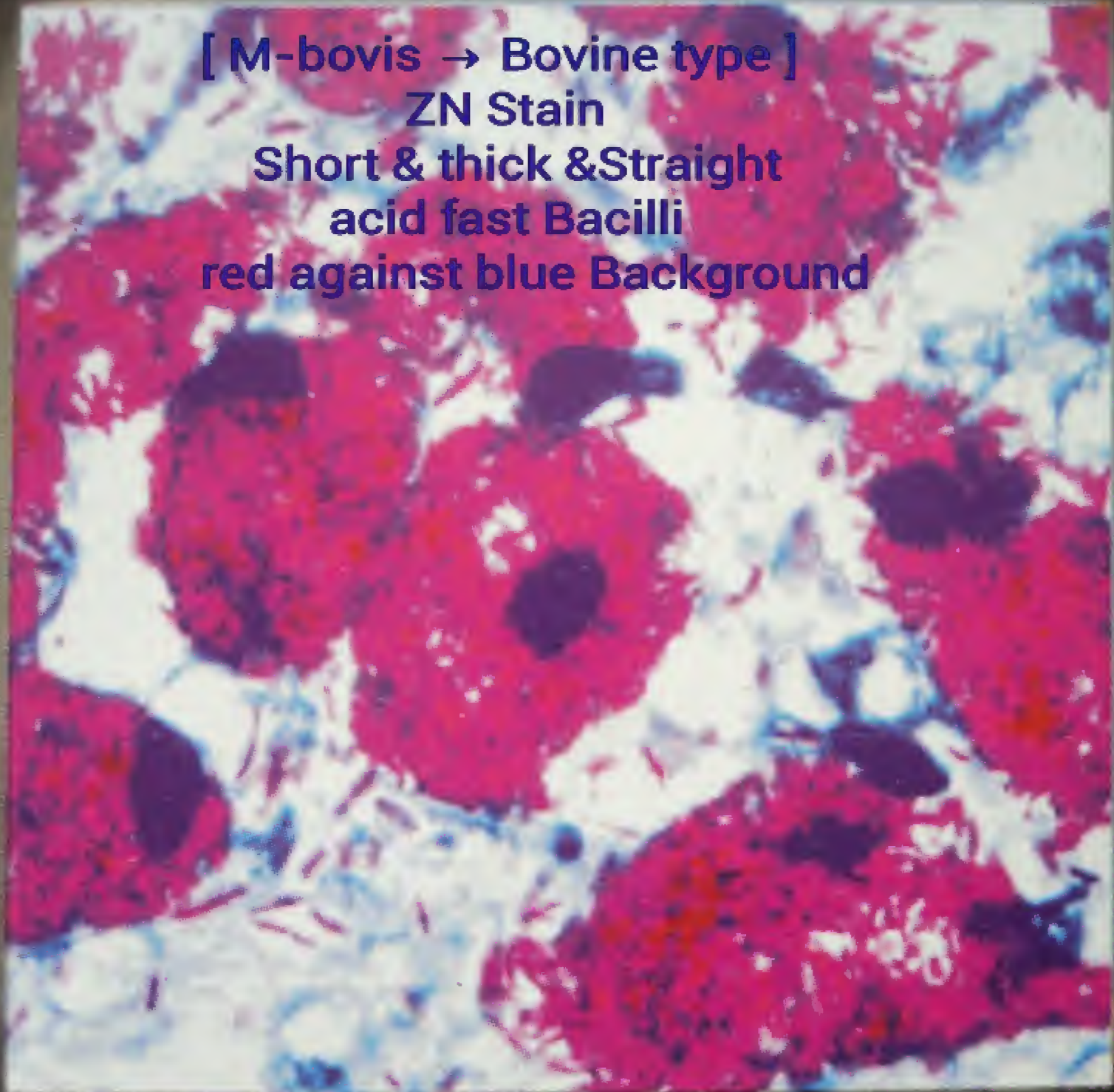
[ M-bovis → Bovine type ]

ZN Stain

Short & thick & Straight

acid fast Bacilli

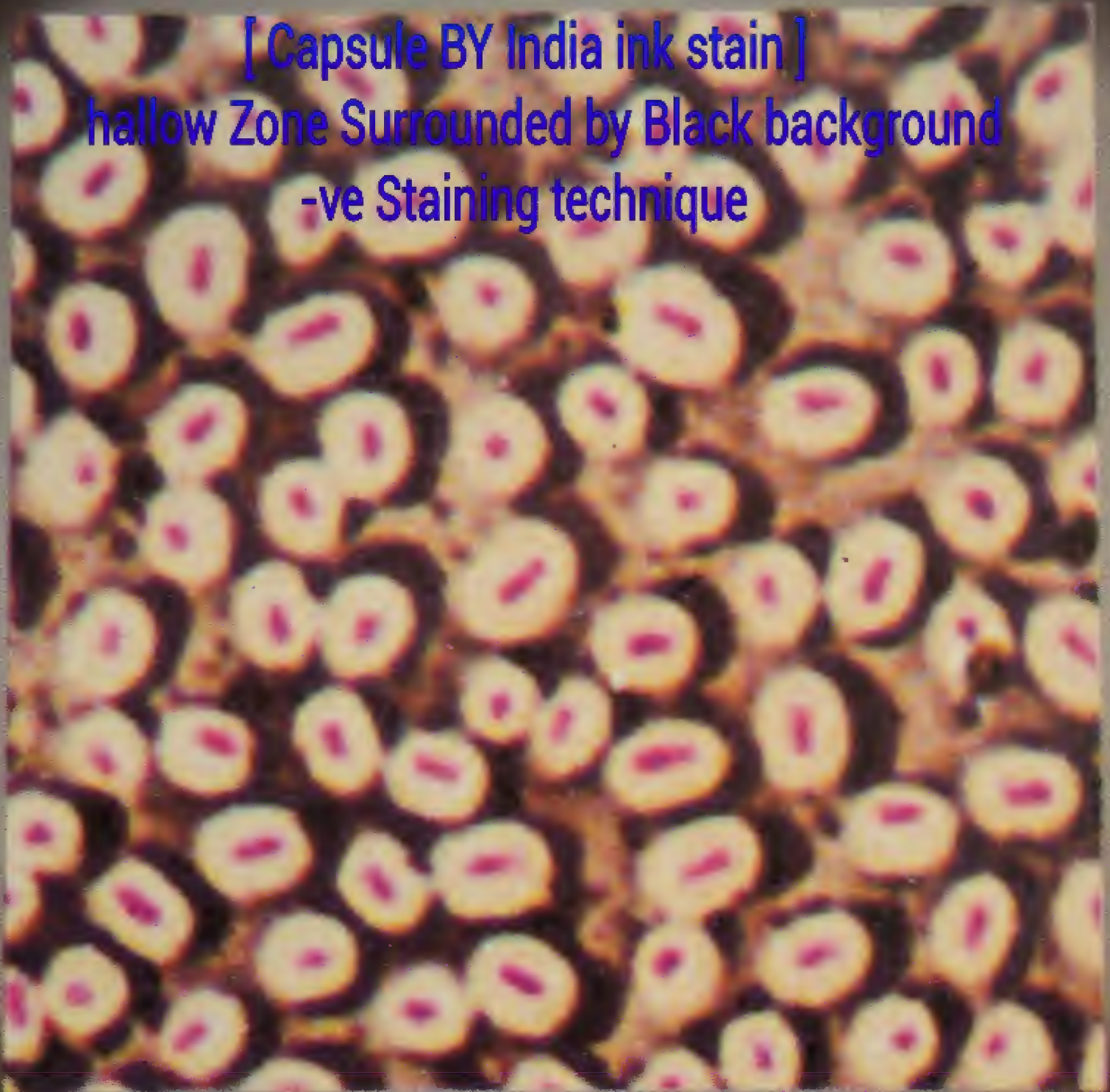
red against blue Background



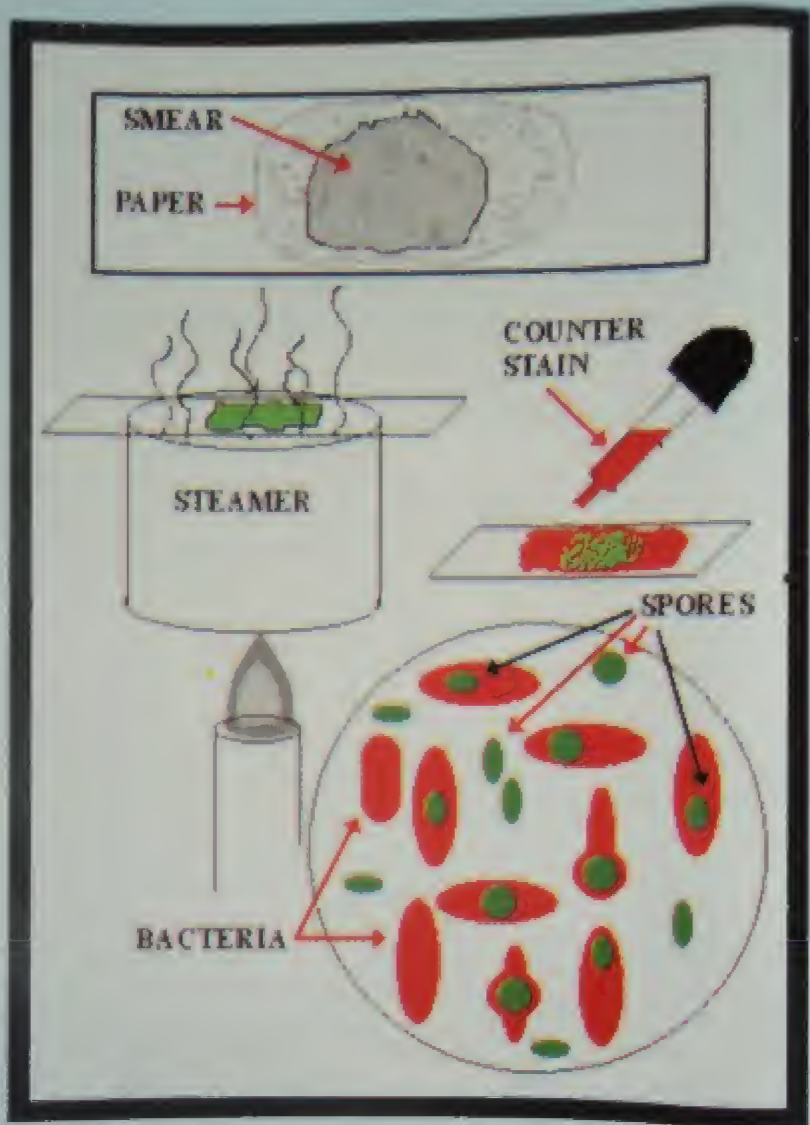
[ Capsule BY India ink stain ]

hallow Zone Surrounded by Black background

-ve Staining technique



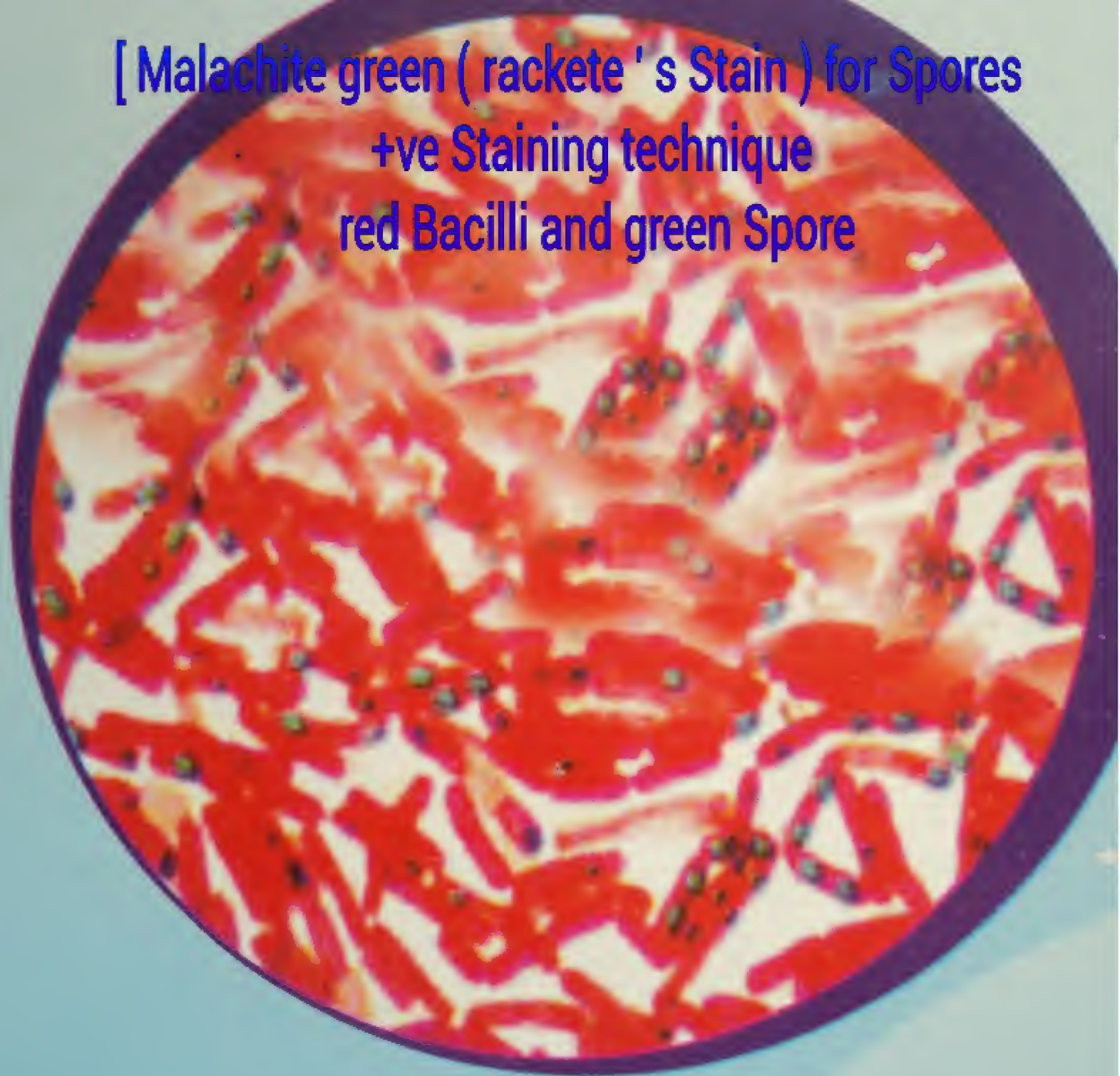




**Steps of staining by  
Malachite green  
(Rachetes's stain)  
for spores**



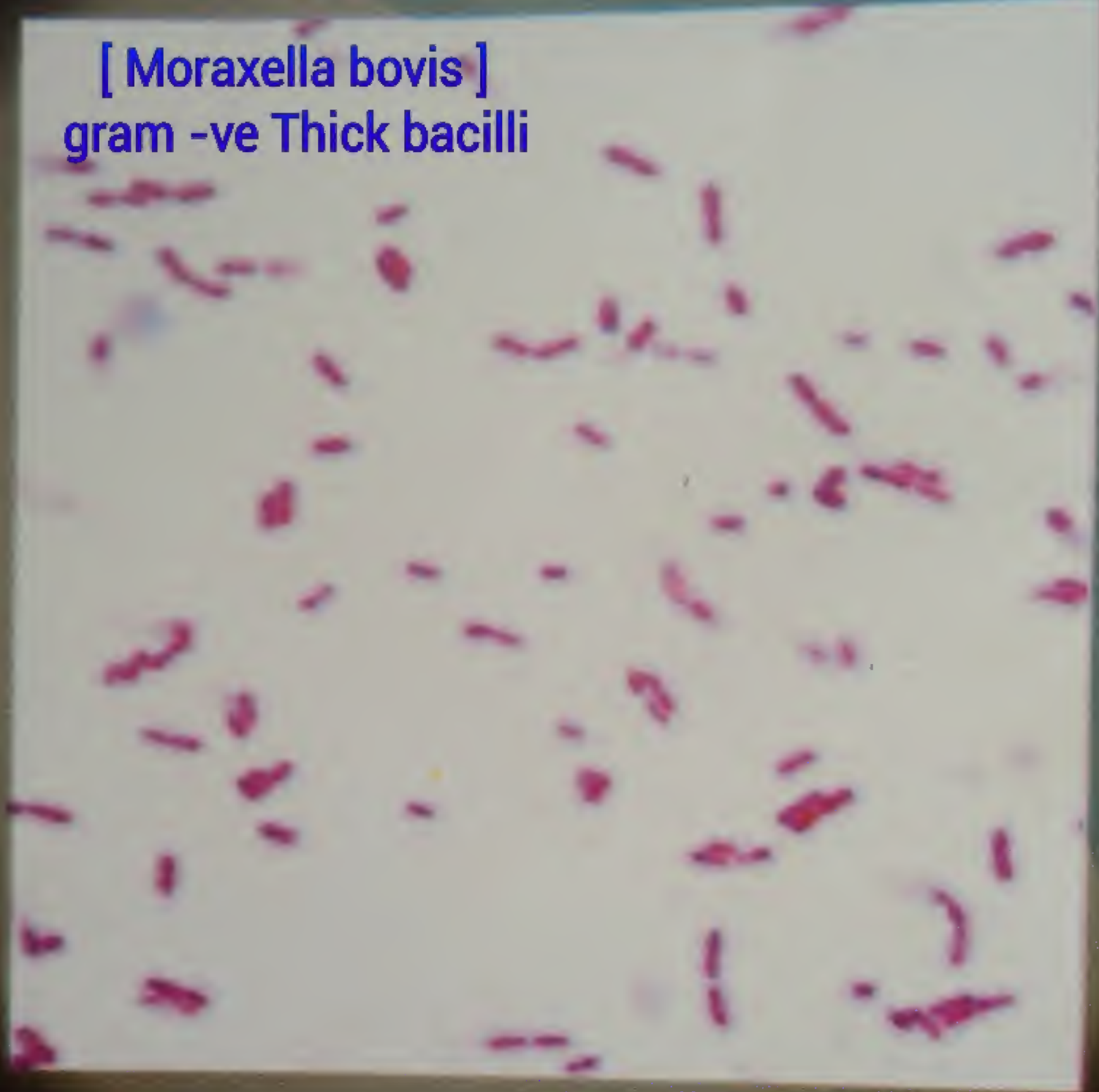
[ Malachite green ( rackete ' s Stain ) for Spores  
+ve Staining technique  
red Bacilli and green Spore



[ *Pseudomonas aeruginosa* ]  
gram -ve Cocco Bacilli

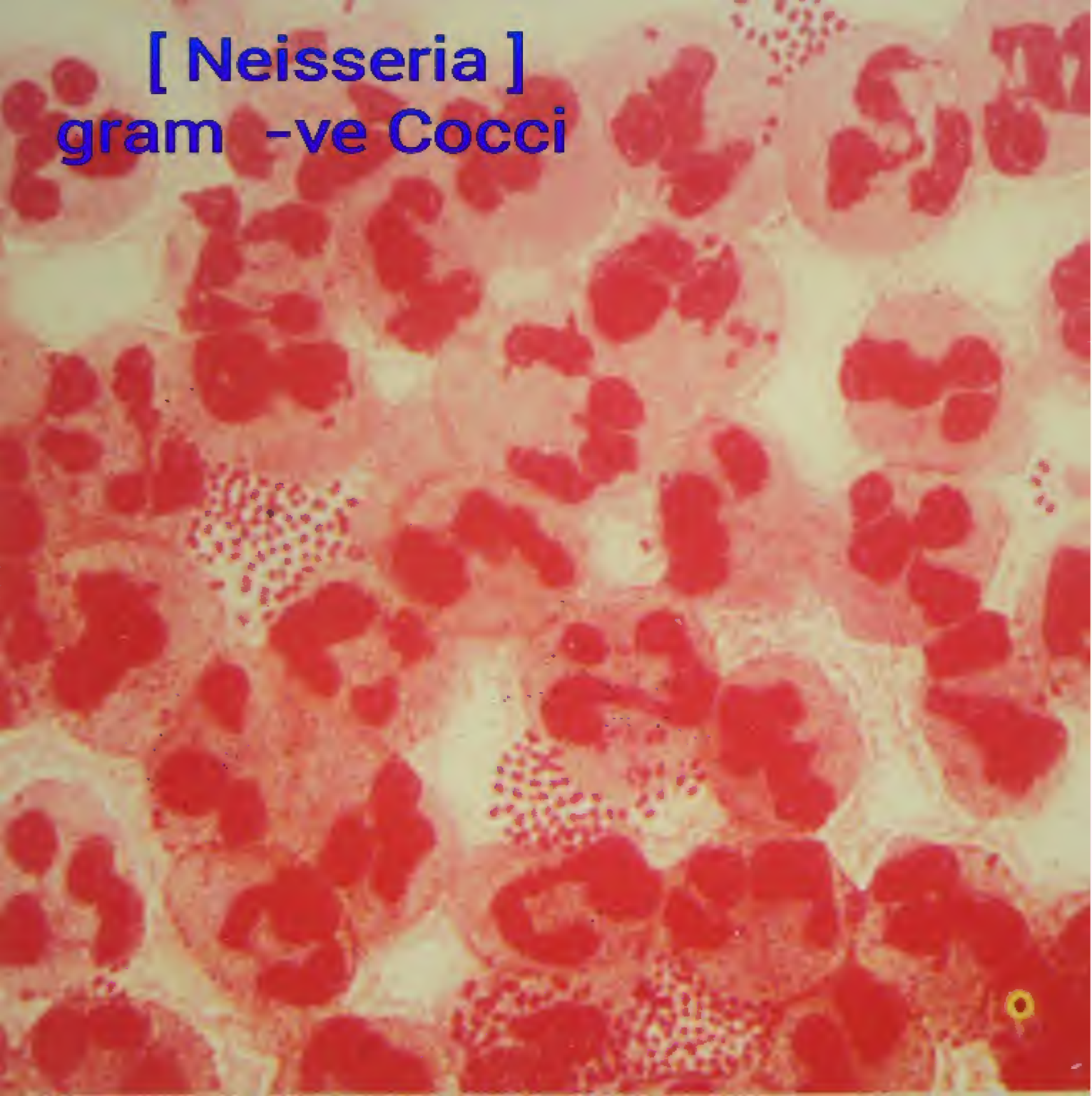


[ *Moraxella bovis* ]  
gram -ve Thick bacilli

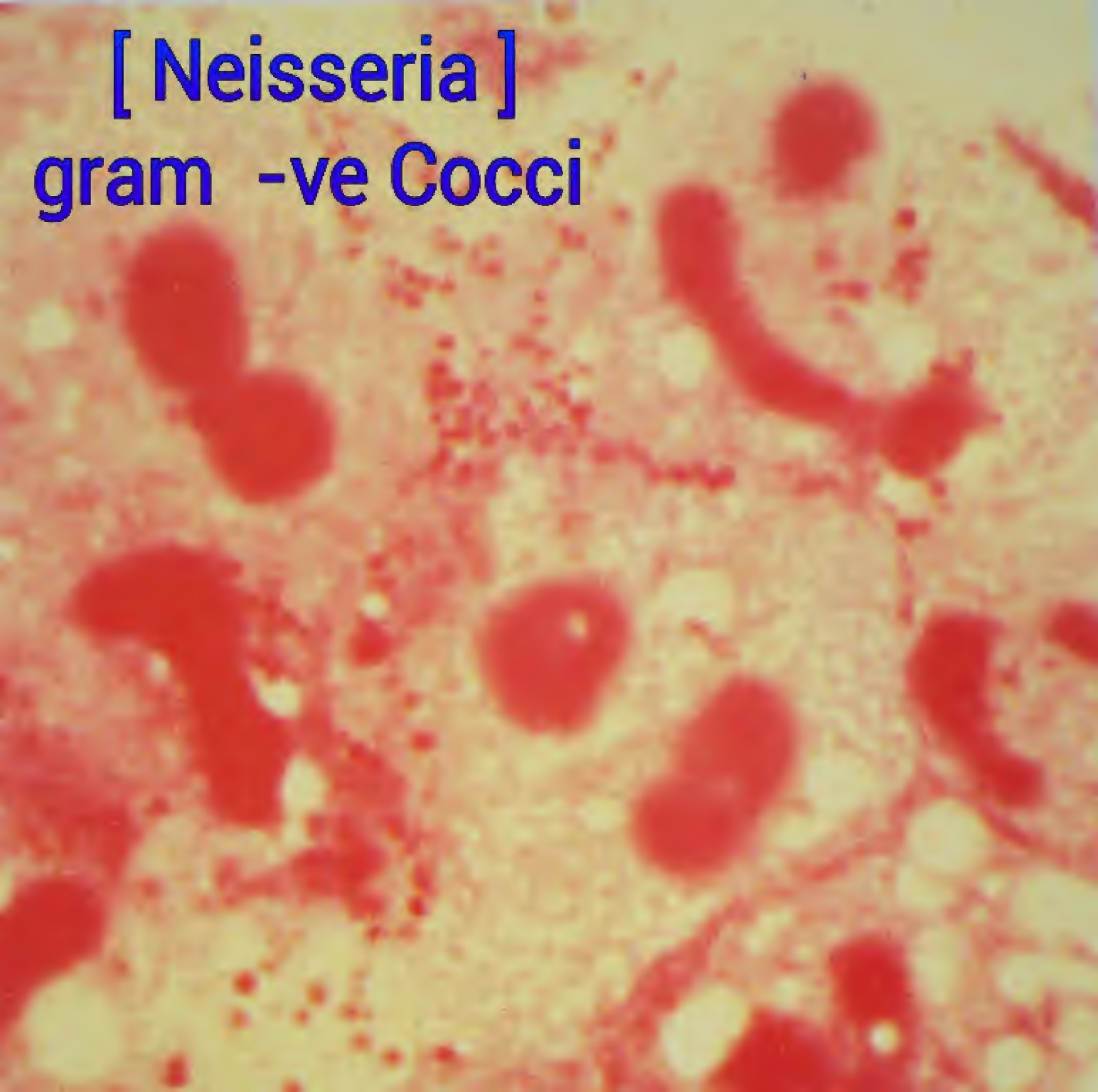




[ *Neisseria* ]  
gram -ve Cocci

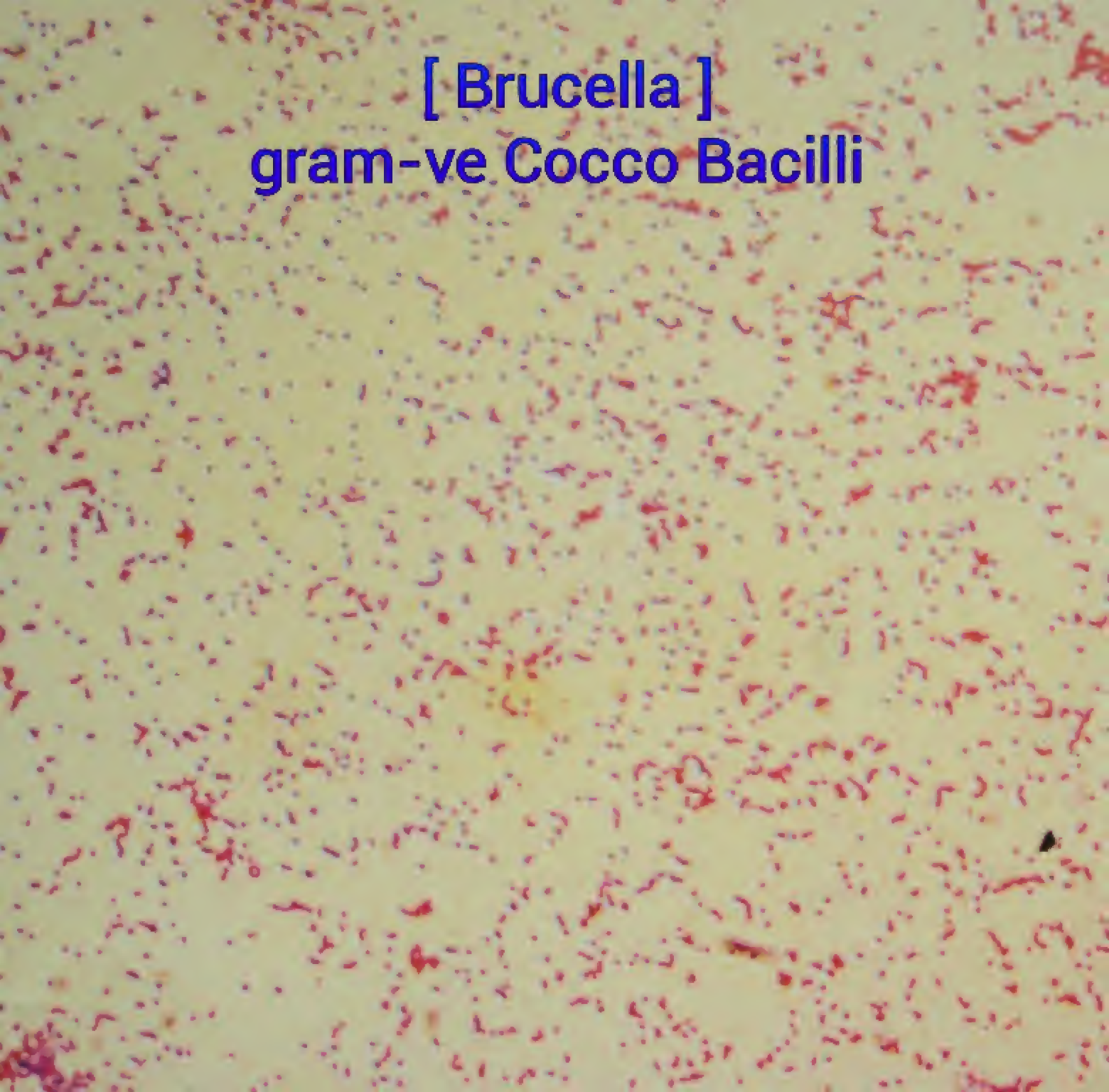


[ Neisseria ]  
gram -ve Cocci



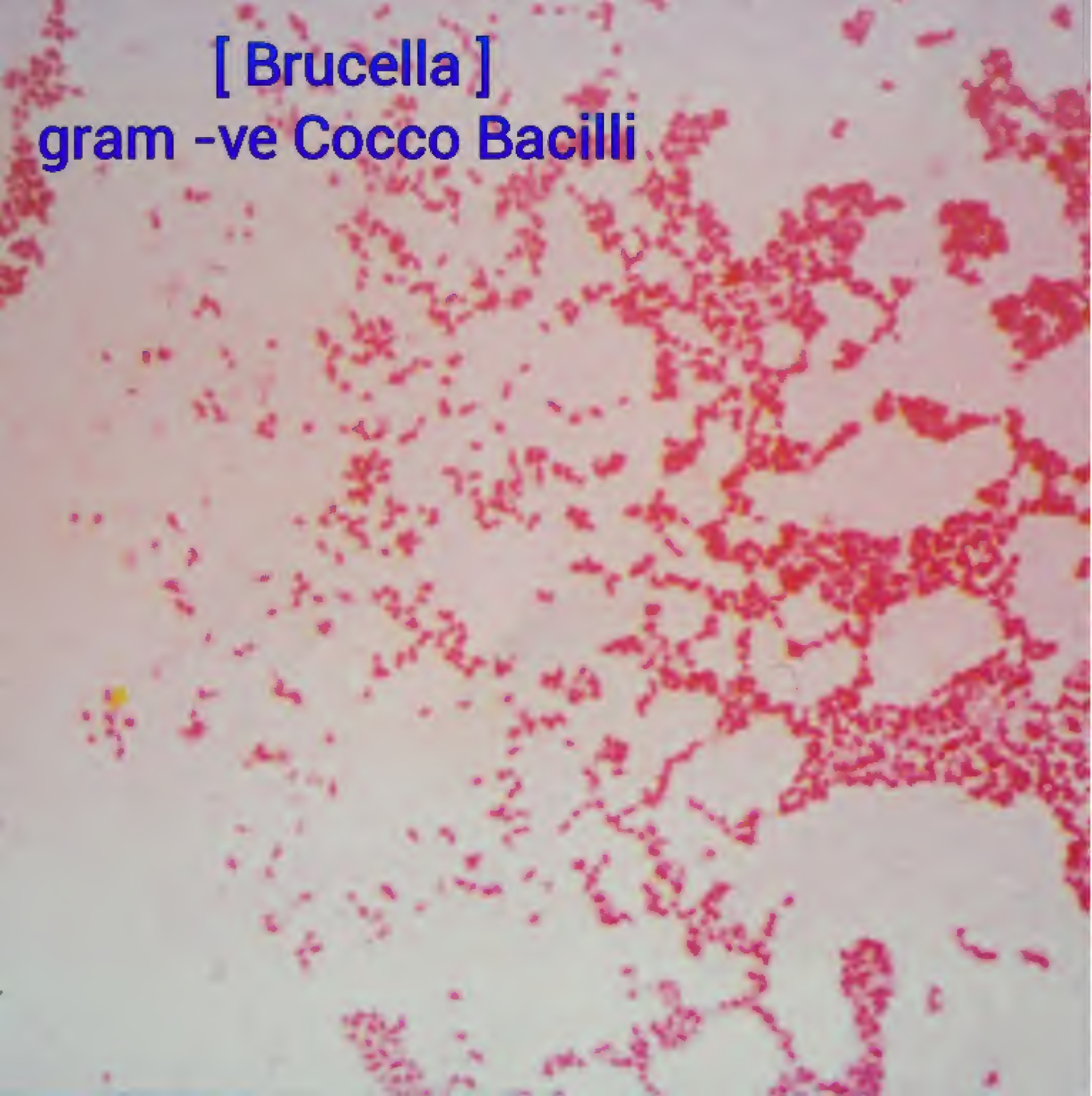


**[ Brucella ]**  
**gram-ve Cocco Bacilli**



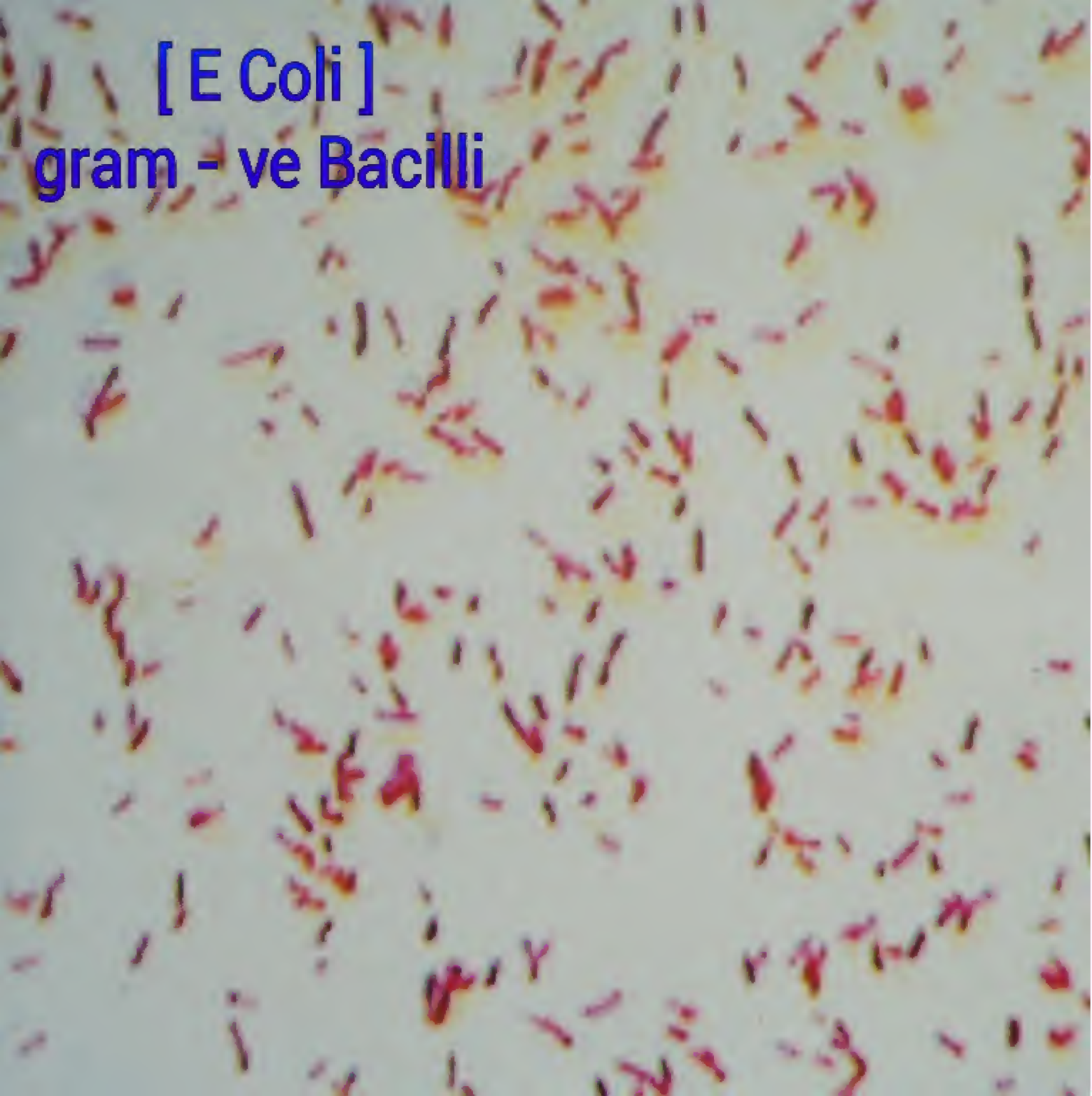


[ Brucella ]  
gram -ve Cocco Bacilli



[ E Coli ]

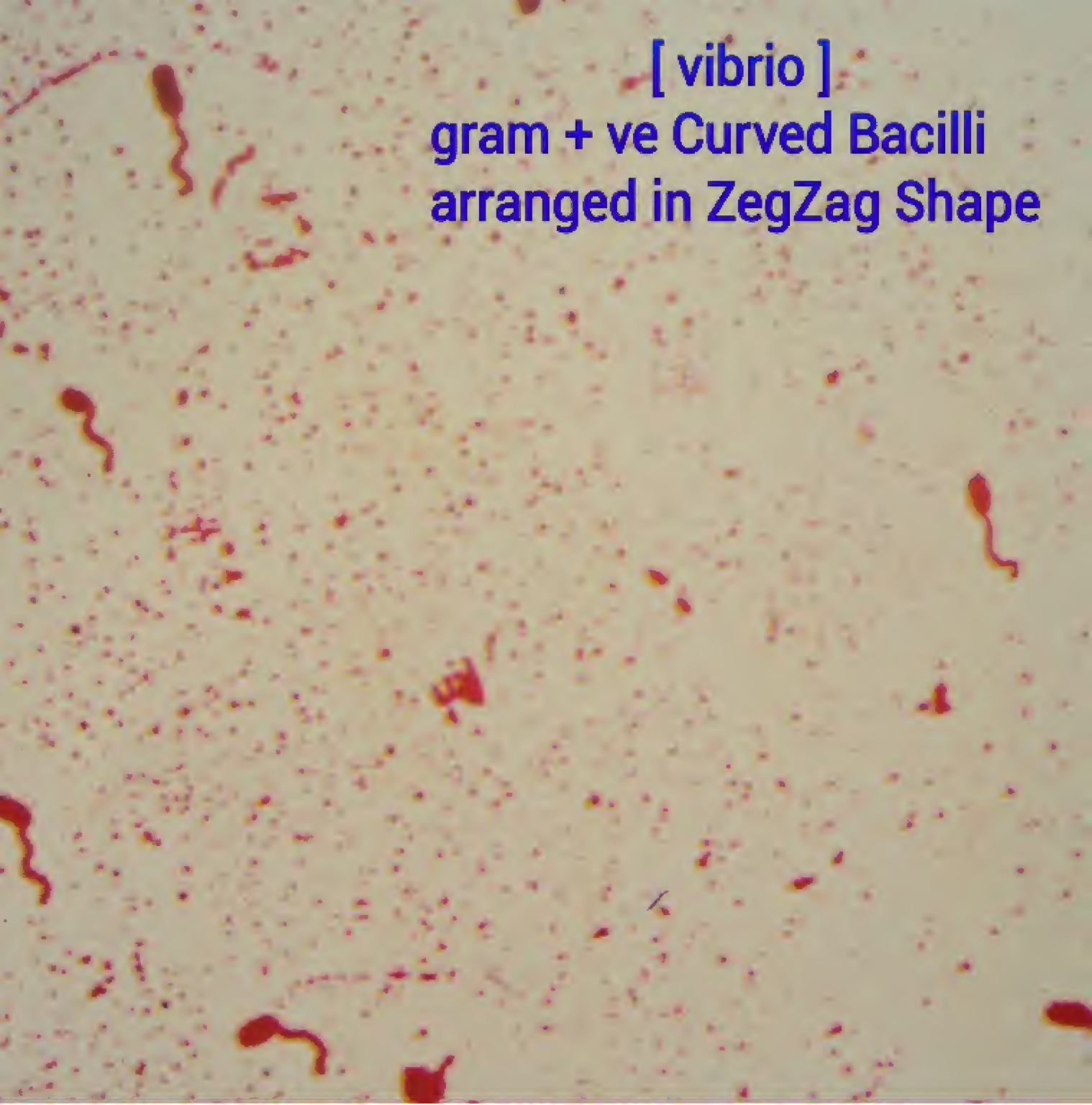
gram - ve Bacilli



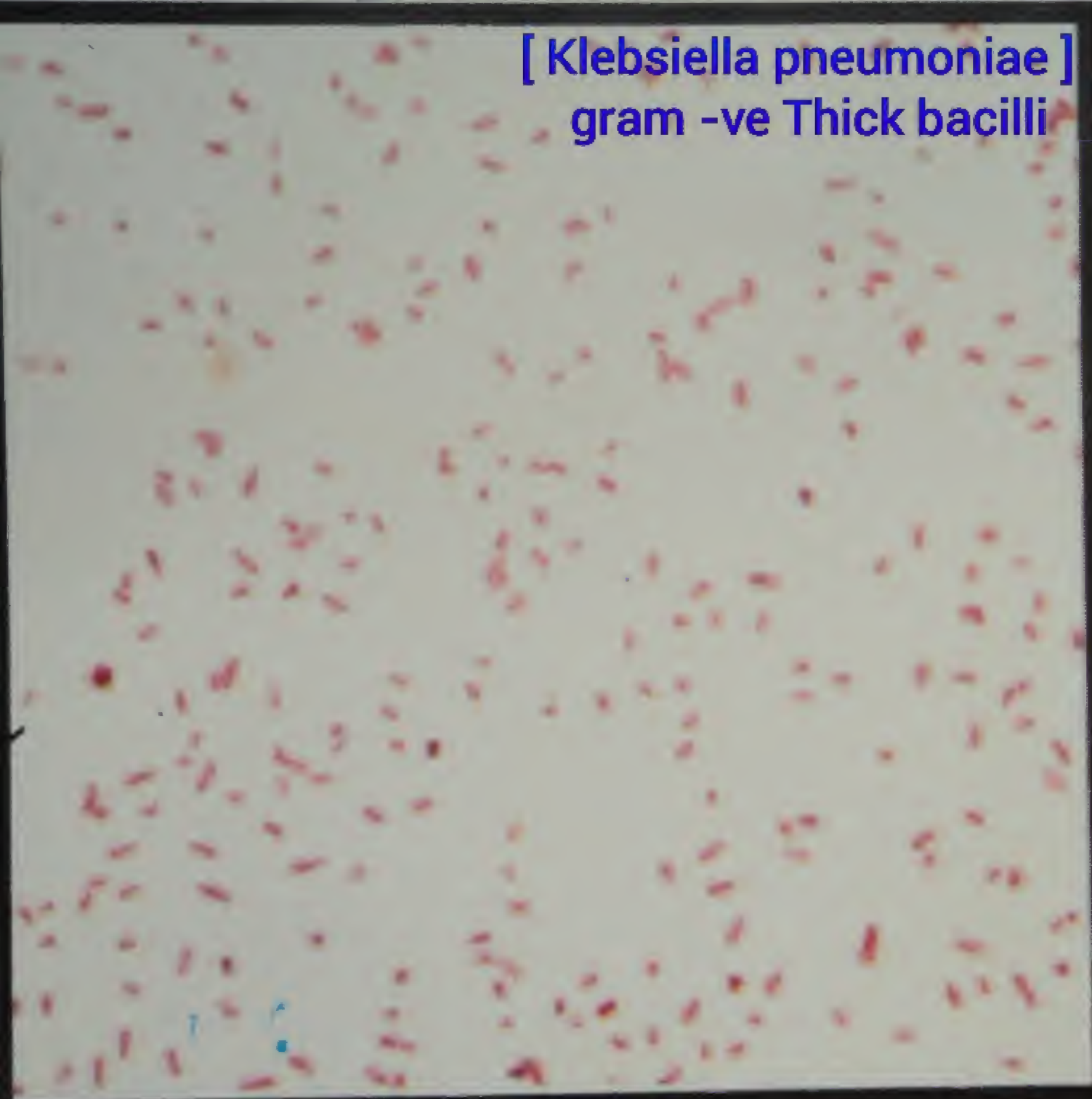


[ vibrio ]

gram + ve Curved Bacilli  
arranged in ZegZag Shape



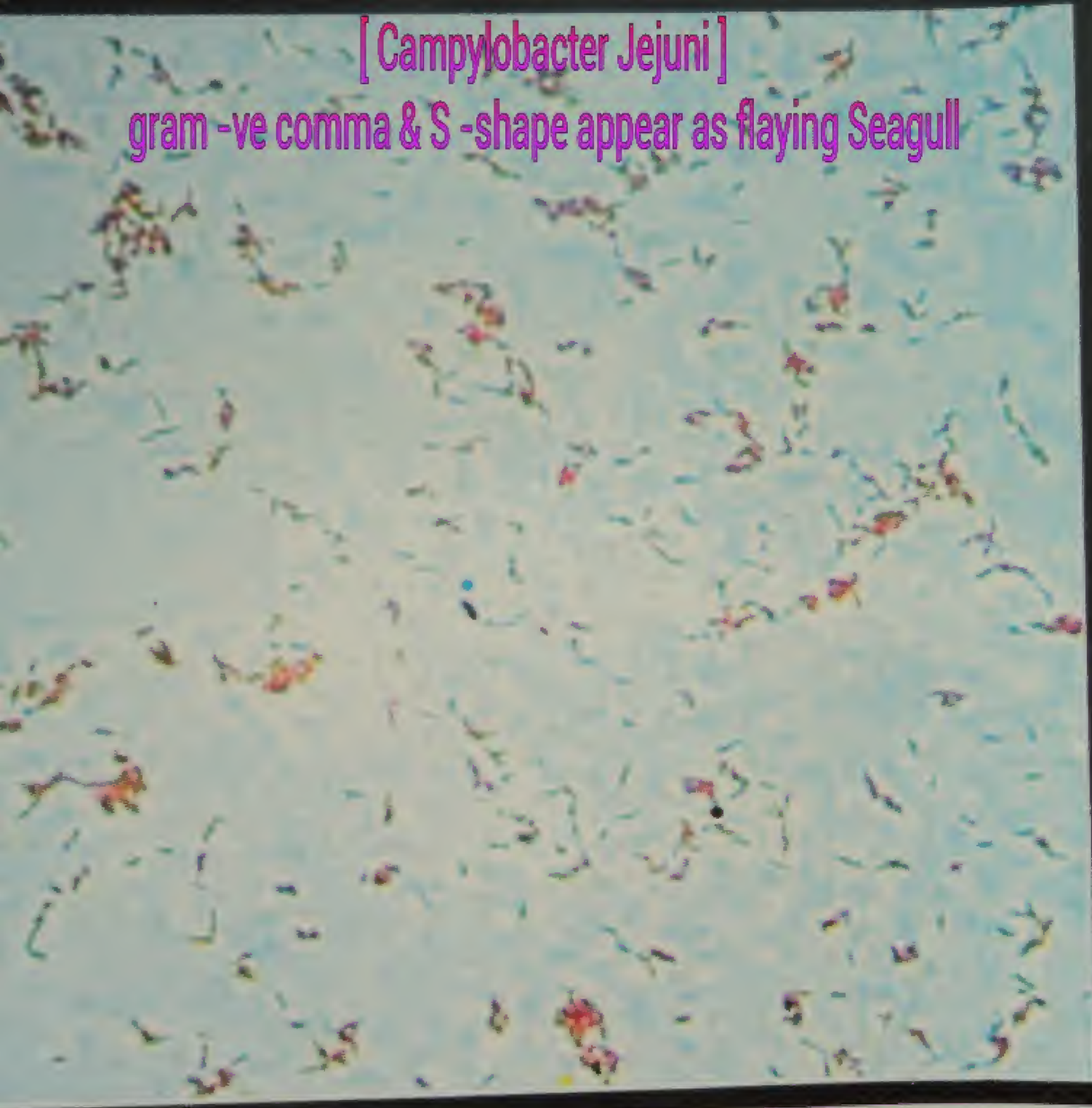
[ *Klebsiella pneumoniae* ]  
gram -ve Thick bacilli





[ *Campylobacter Jejuni* ]

gram -ve comma & S -shape appear as flaying Seagull



[ yersinia Pestis-Plague ]  
gram-ve Bacilli with bipolar appearance

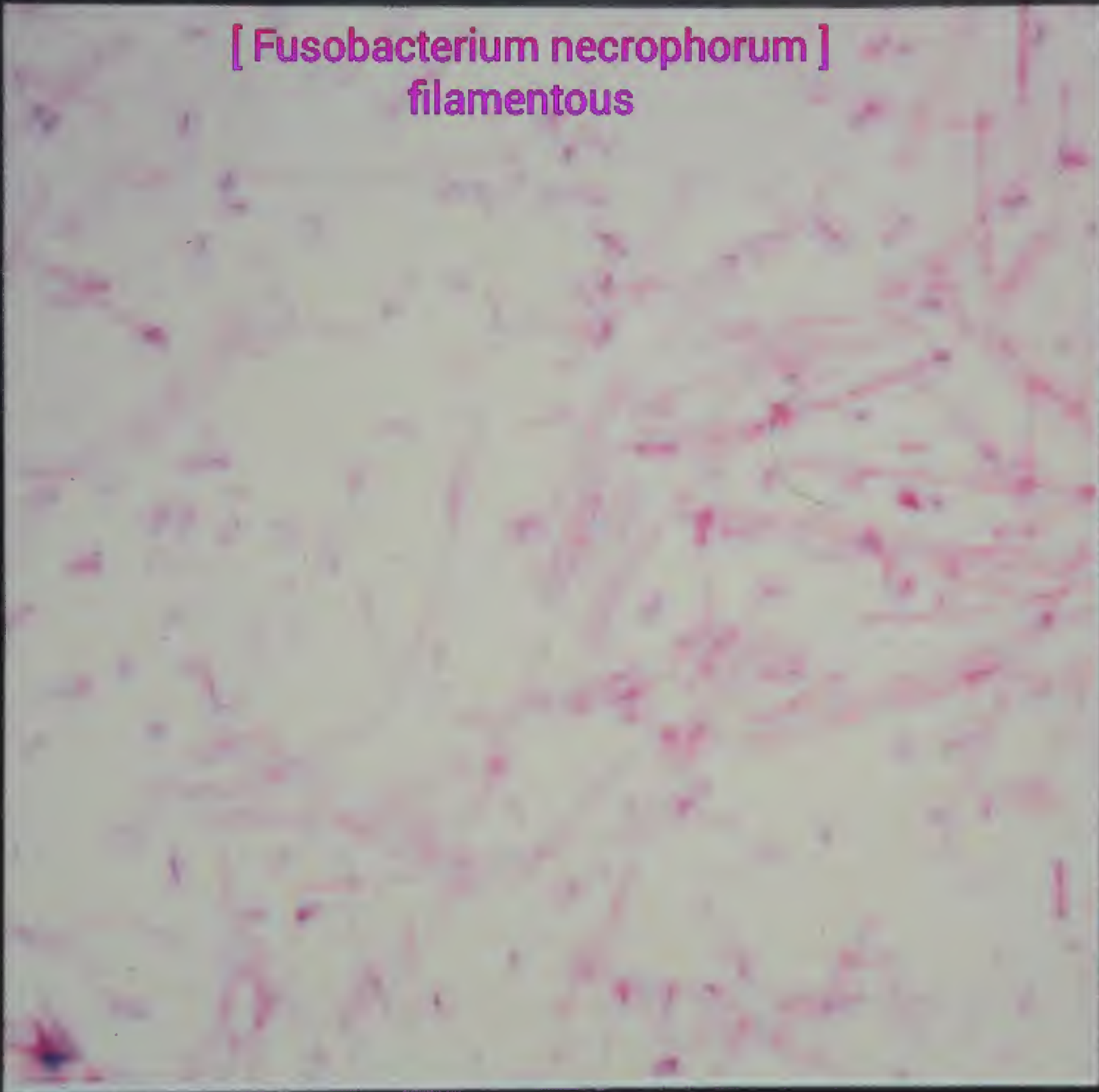


[ yersinia Pseudotuberculosis ]  
gram -ve Bacilli

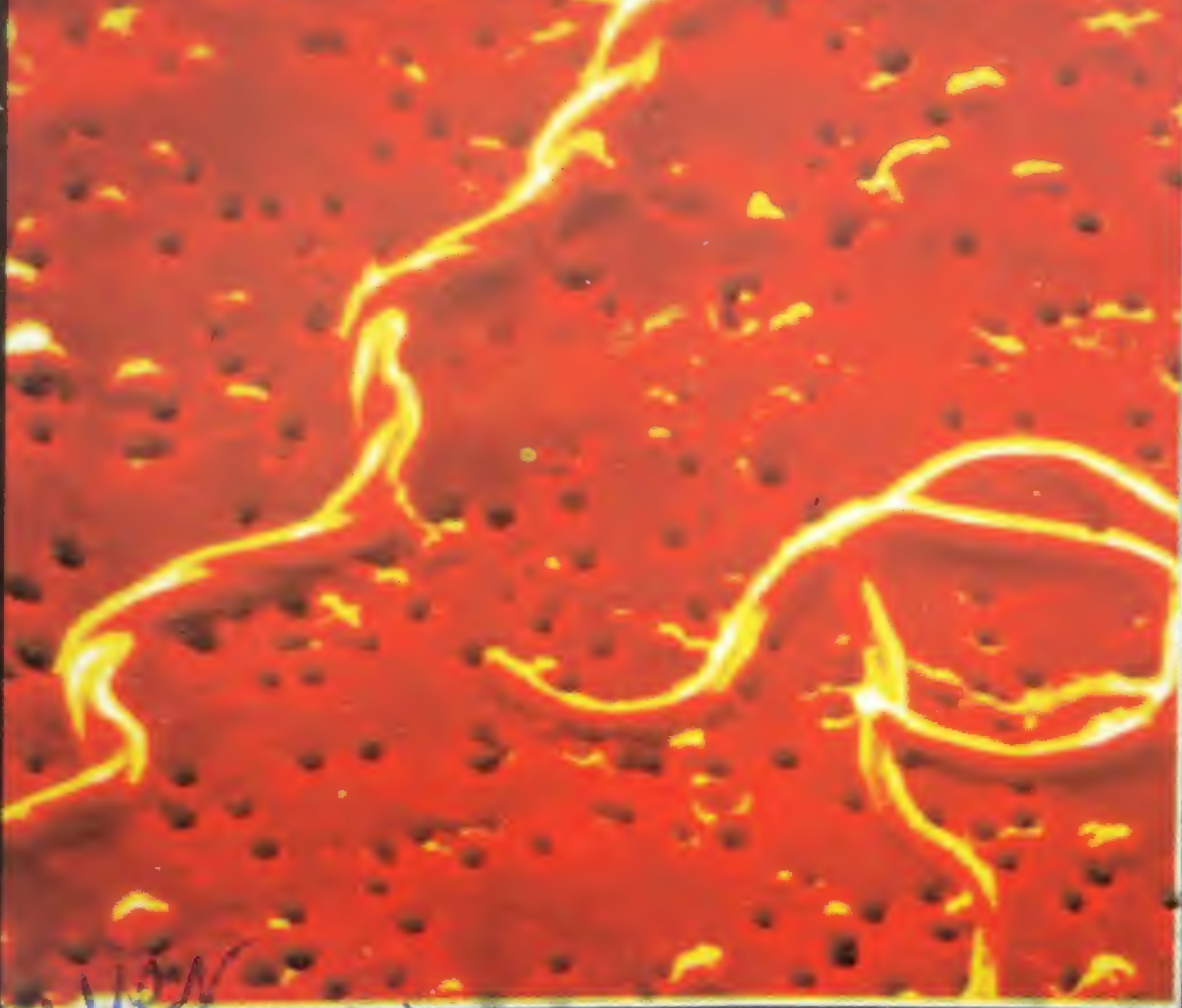




[ *Fusobacterium necrophorum* ]  
filamentous



[ leptospira by Silver impregnation Stain ]  
Thin spiral with Crack screw hooked end



[ leptospira by Phase contrast microscope ]  
Thin spiral with Crack screw hooked end





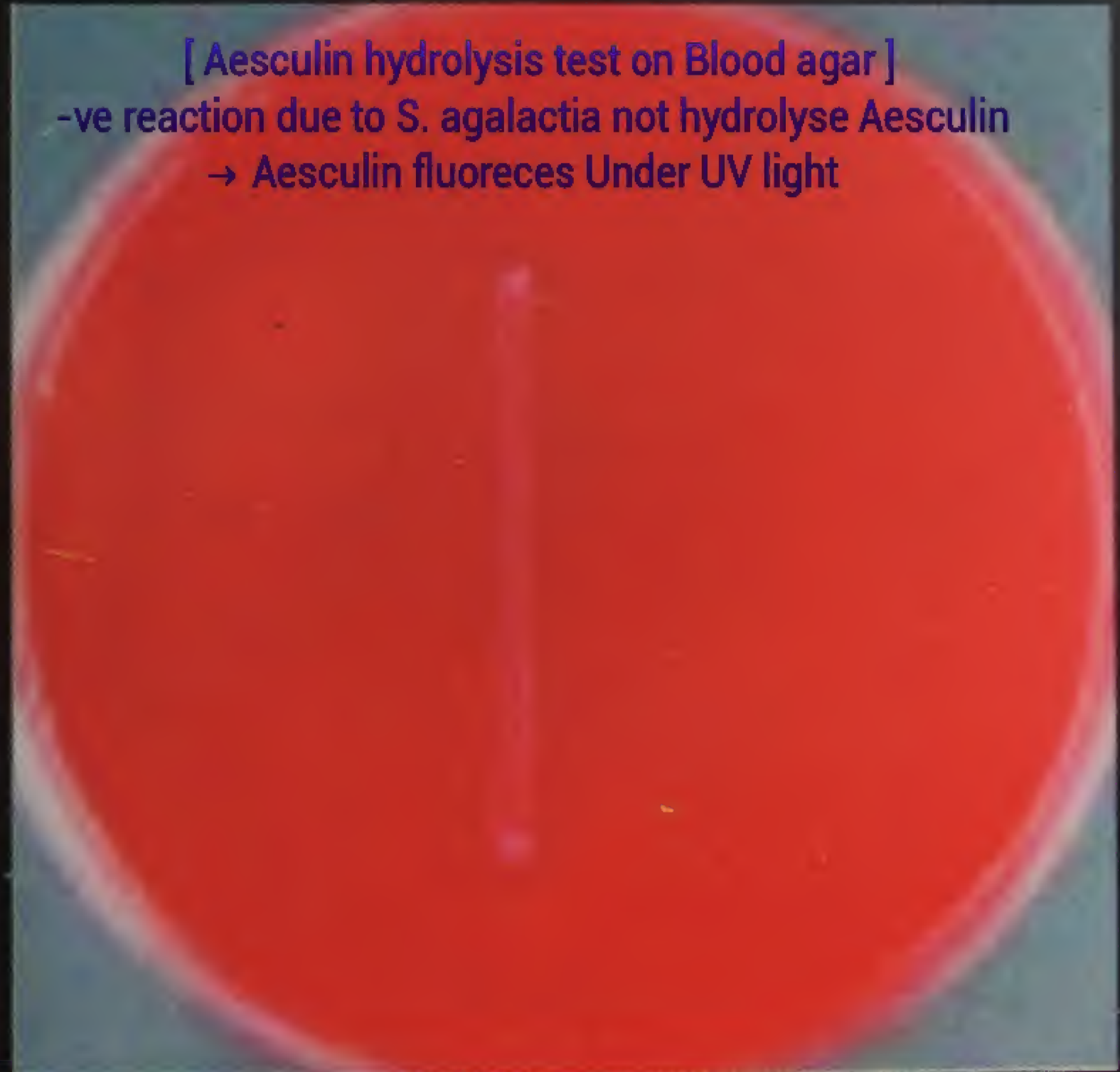


[ Borrelia ]

Thick spiral bacteria

**[ Aesculin hydrolysis test on Blood agar ]**

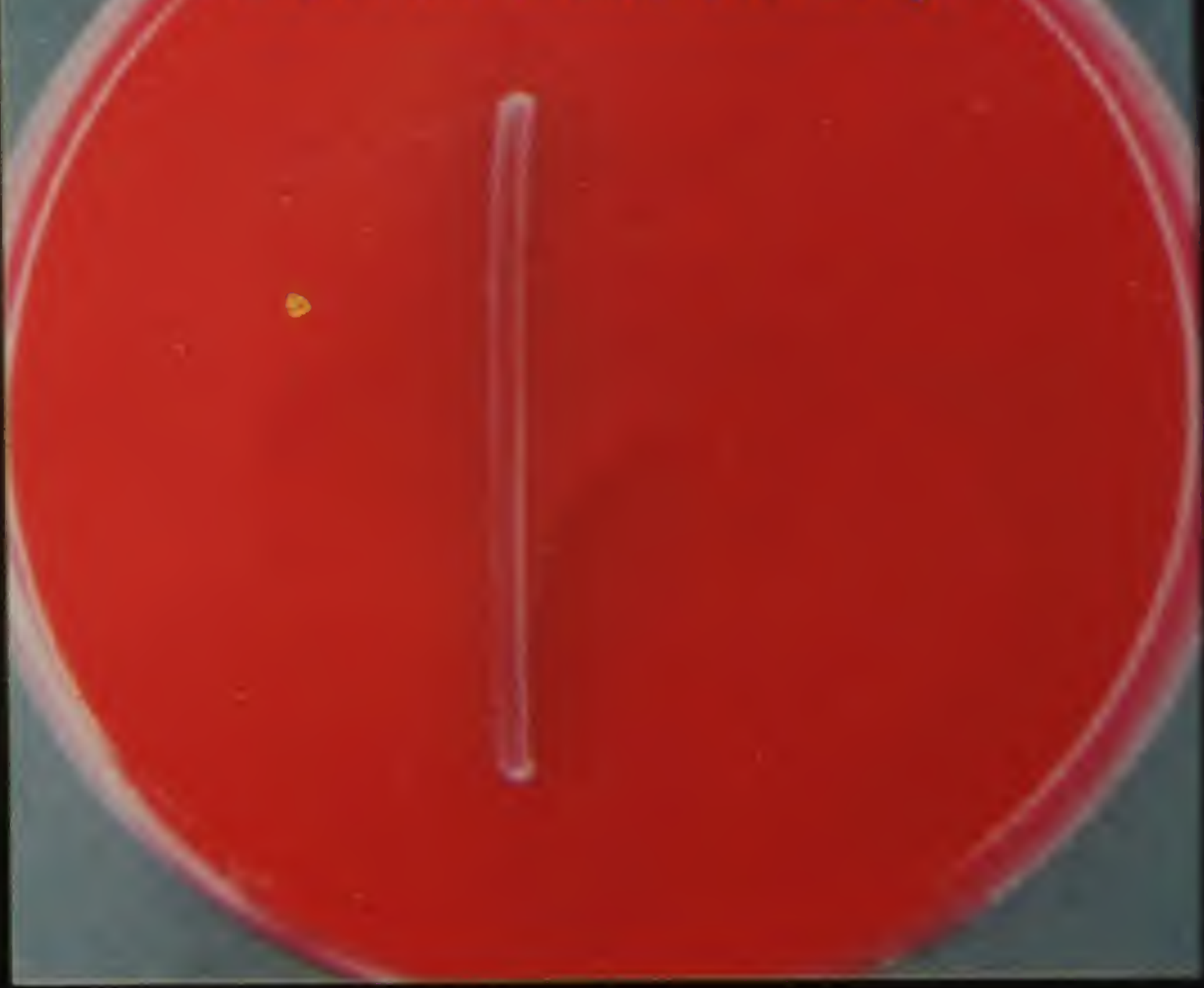
- ve reaction due to *S. agalactia* not hydrolyse Aesculin
- Aesculin fluoresces Under UV light



[ Aesculin hydrolysis test on Blood agar ]

+Ve reaction due to *E. faecalis* hydrolyse Aesculin

→ Aesculin not fluoresces under UV light





**[ lecithinase test ]**

**Media Containing egg Yolk**

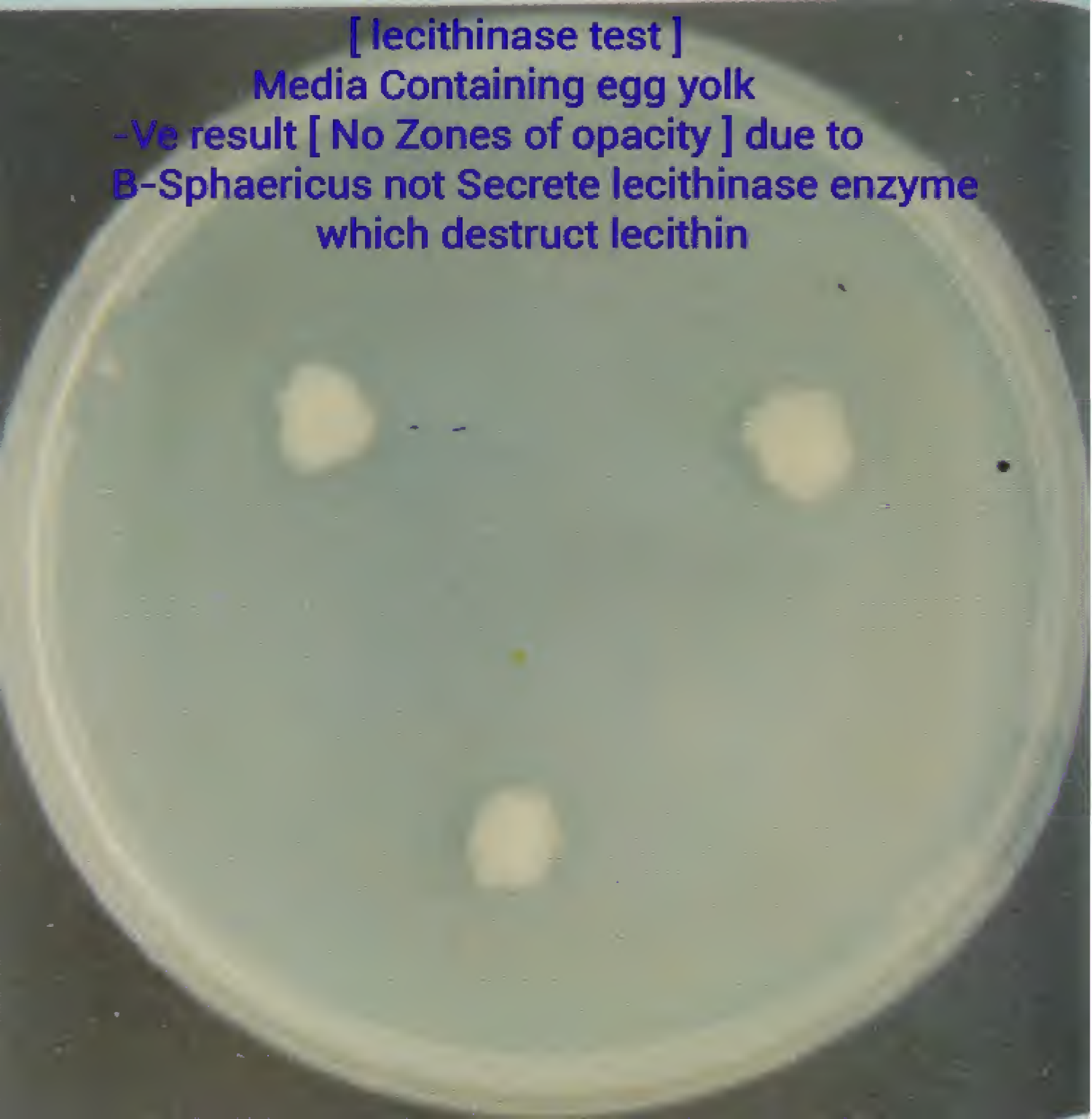
**+ ve result [ Zones of opacity ] by B-Cereus  
which Secretes lecithinase enzyme which  
destruct protein**



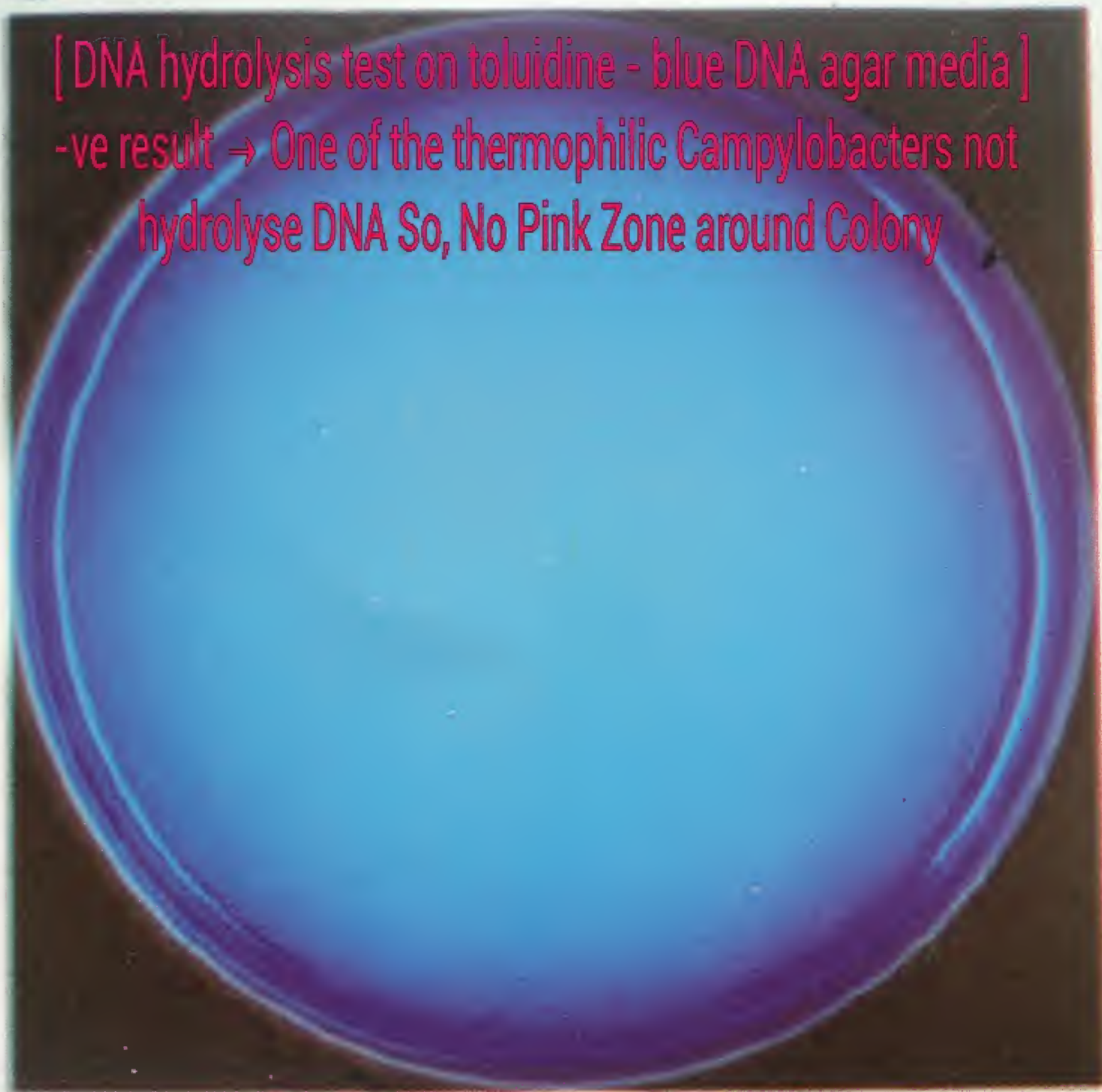
[ lecithinase test ]

Media Containing egg yolk

-Ve result [ No Zones of opacity ] due to  
B-Sphaericus not Secrete lecithinase enzyme  
which destruct lecithin

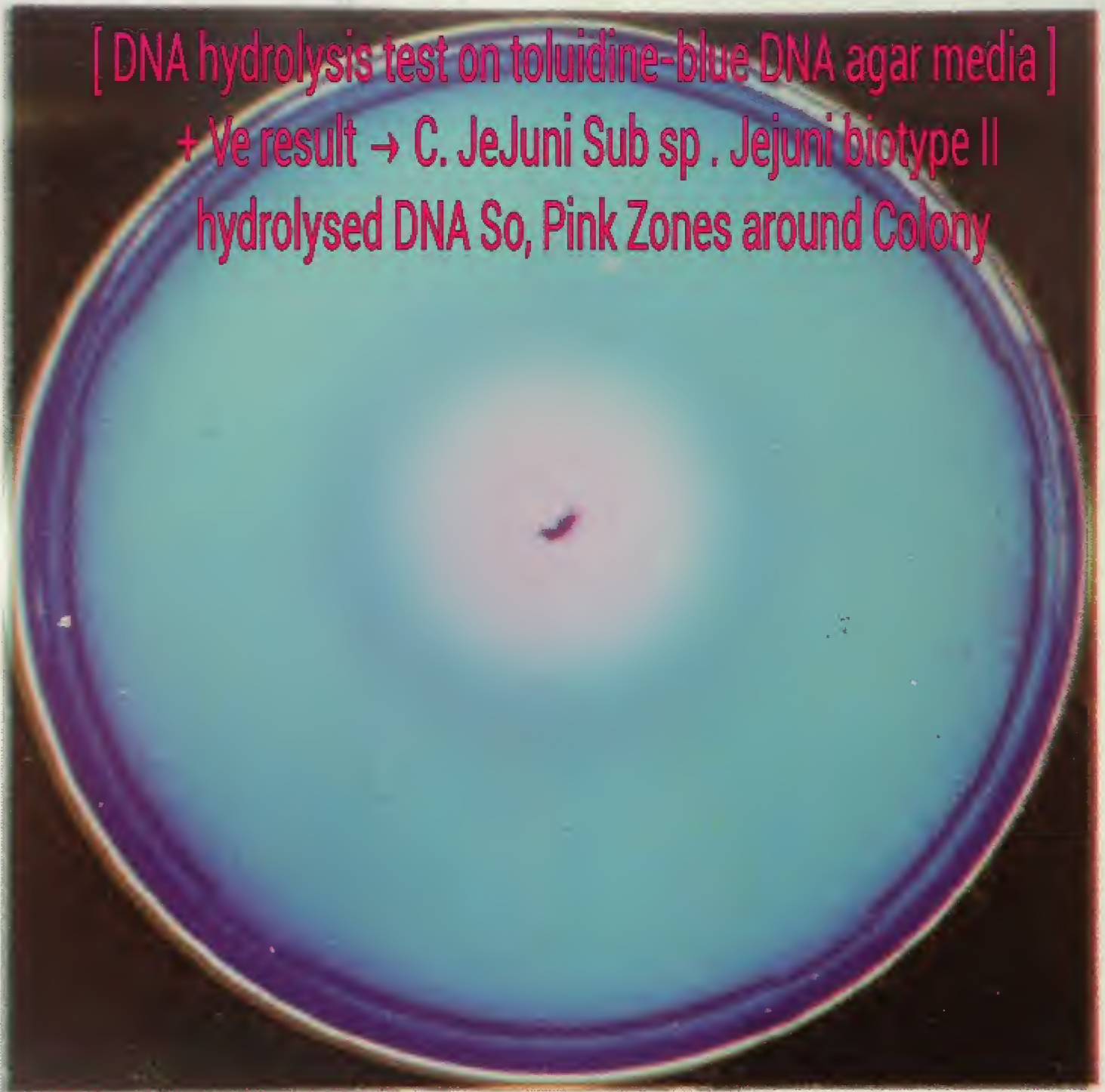


[ DNA hydrolysis test on toluidine - blue DNA agar media ]  
-ve result → One of the thermophilic Campylobacters not  
hydrolyse DNA So, No Pink Zone around Colony





[ DNA hydrolysis test on toluidine-blue DNA agar media ]  
+ Ve result → C. JeJuni Sub sp . Jejuni biotype II  
hydrolysed DNA So, Pink Zones around Colony



[ gelatin hydrolysis test ]

indicator → Sulpho salicylic acid

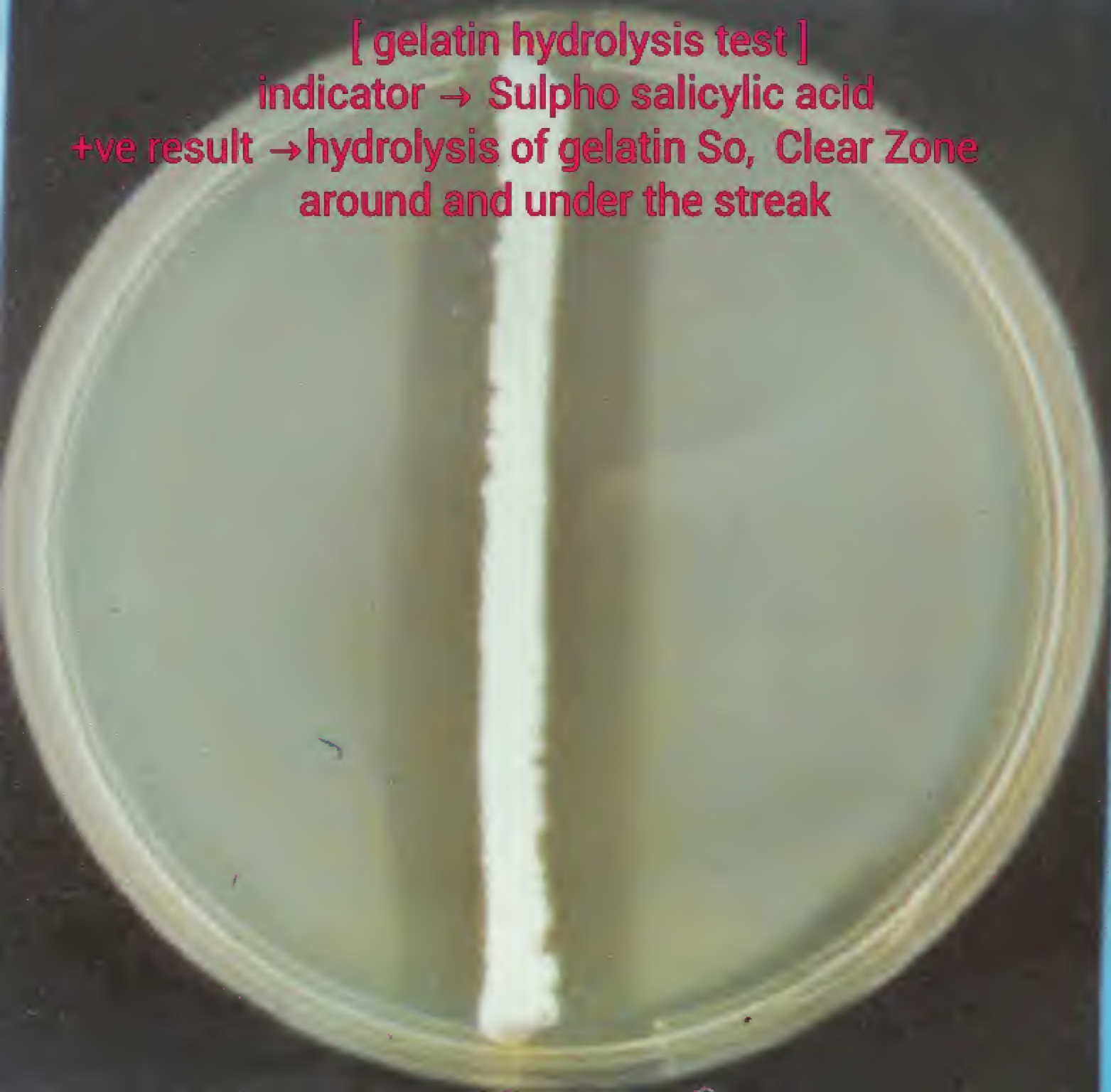
-ve result → no hydrolysis of gelatin So, no Clear Zone  
around and under the streak



[ gelatin hydrolysis test ]

indicator → Sulpho salicylic acid

+ve result → hydrolysis of gelatin So, Clear Zone  
around and under the streak

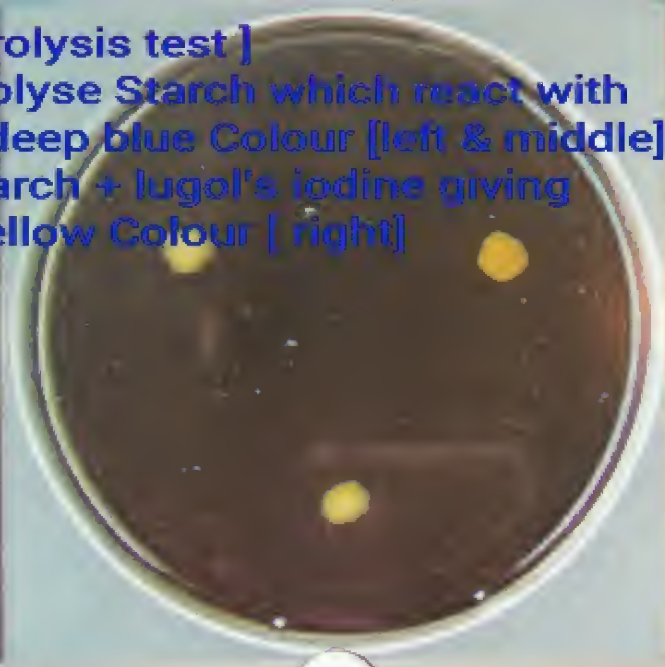
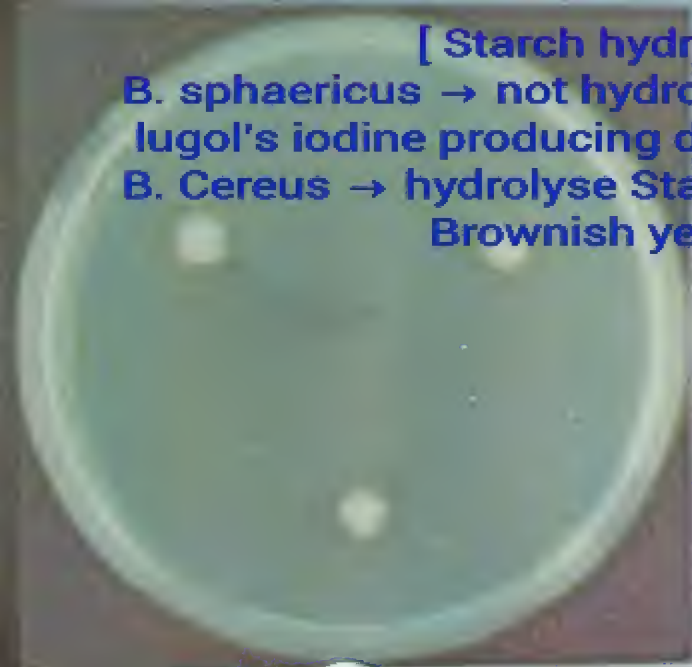




**[ Starch hydrolysis test ]**

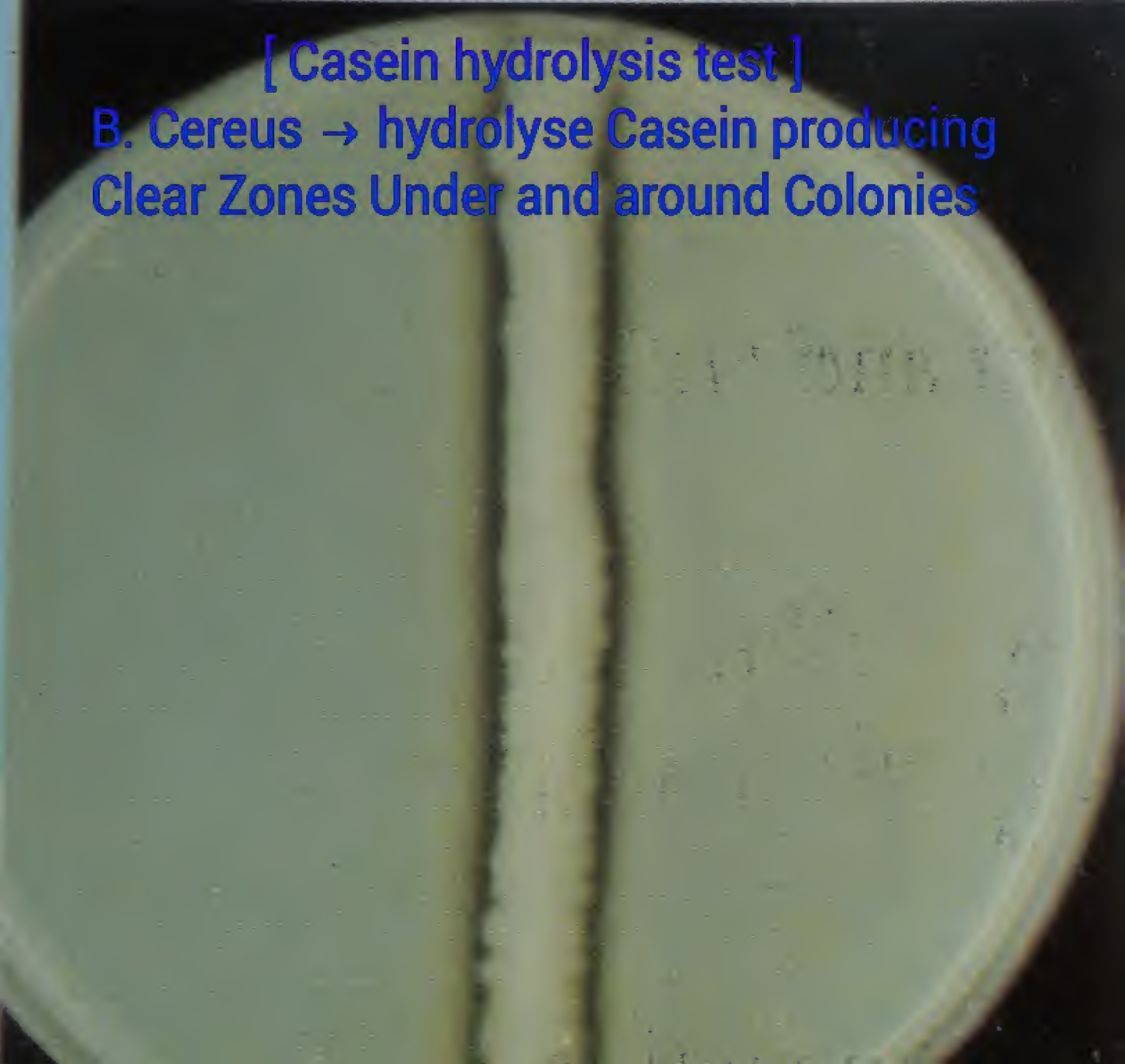
**B. sphaericus → not hydrolyse Starch which react with lugol's iodine producing deep blue Colour [left & middle]**

**B. Cereus → hydrolyse Starch + lugol's iodine giving Brownish yellow Colour [ right]**



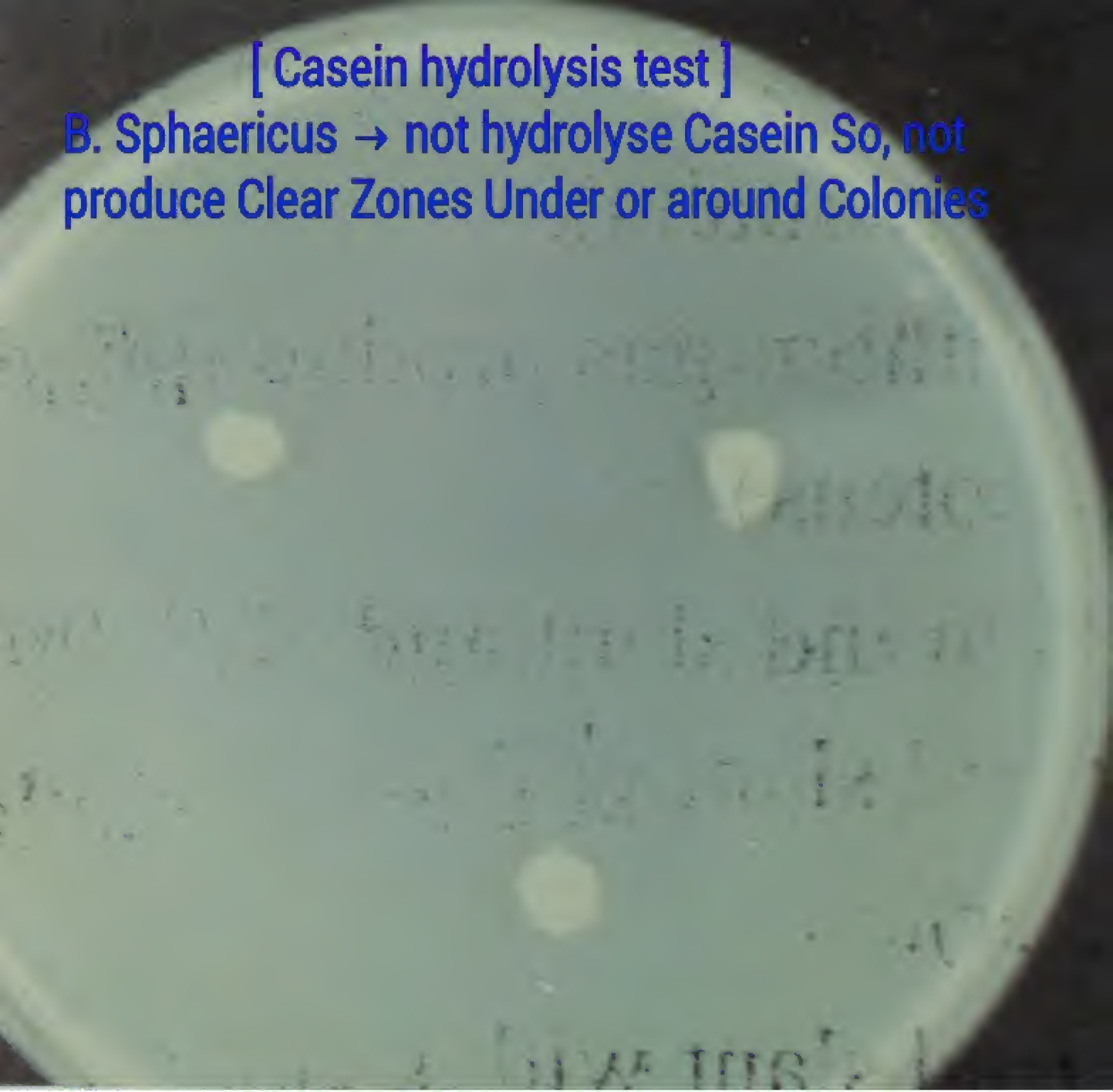
## [ Casein hydrolysis test ]

**B. Cereus → hydrolyse Casein producing  
Clear Zones Under and around Colonies**



## [ Casein hydrolysis test ]

**B. Sphaericus** → not hydrolyse Casein So, not produce Clear Zones Under or around Colonies







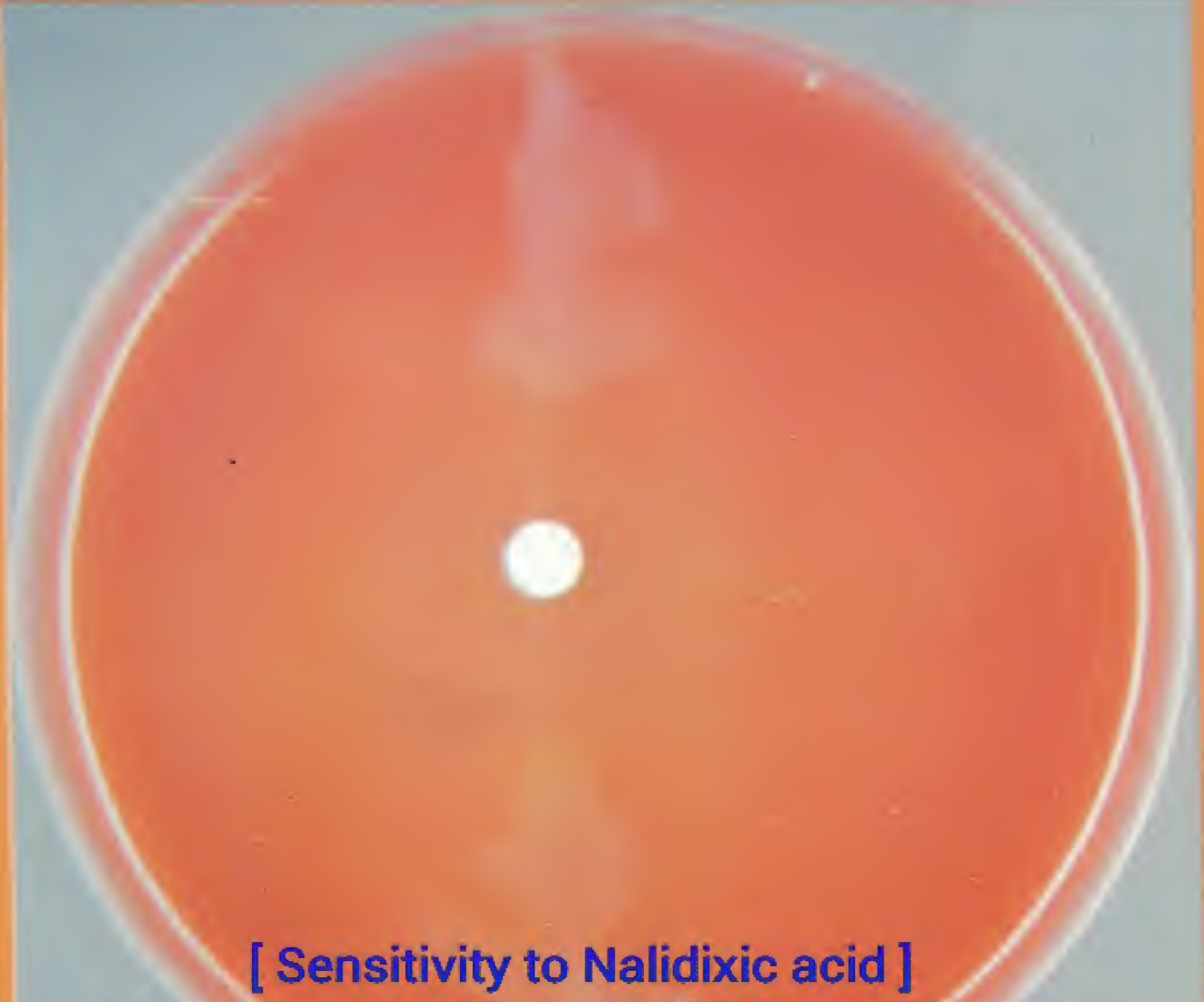
[ CAMP test ]

**S. agalactia produce extracellular Compounds Which Conjugate with Beta haemolysin of S.aureus producing Complete haemolysis of Sheep RBCs on the Blood agar**

[ Satellitism → Satellite test ]

growth of *Haemophilus* around Streak of *S. aureus*  
on X- factor from haemolysed RBCs and V-factor from  
NAD that provided by haemolytic action of *S. aureus* On  
RBCs

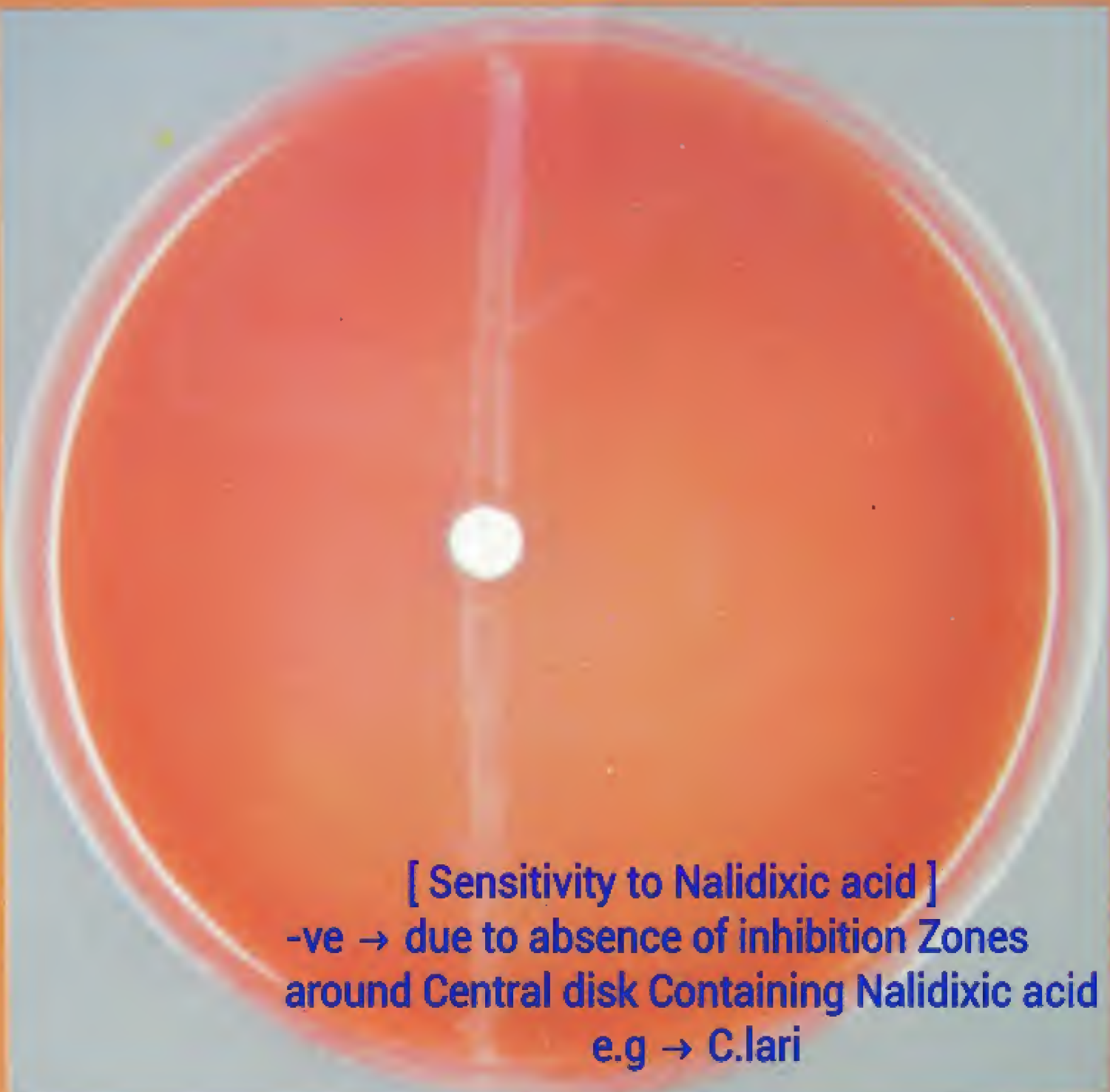




**[ Sensitivity to Nalidixic acid ]**

**+ve → due to presence of inhibition Zones  
around Central disk Containing Nalidixic acid  
e.g → C.Jejuni sub.sp jejuni**

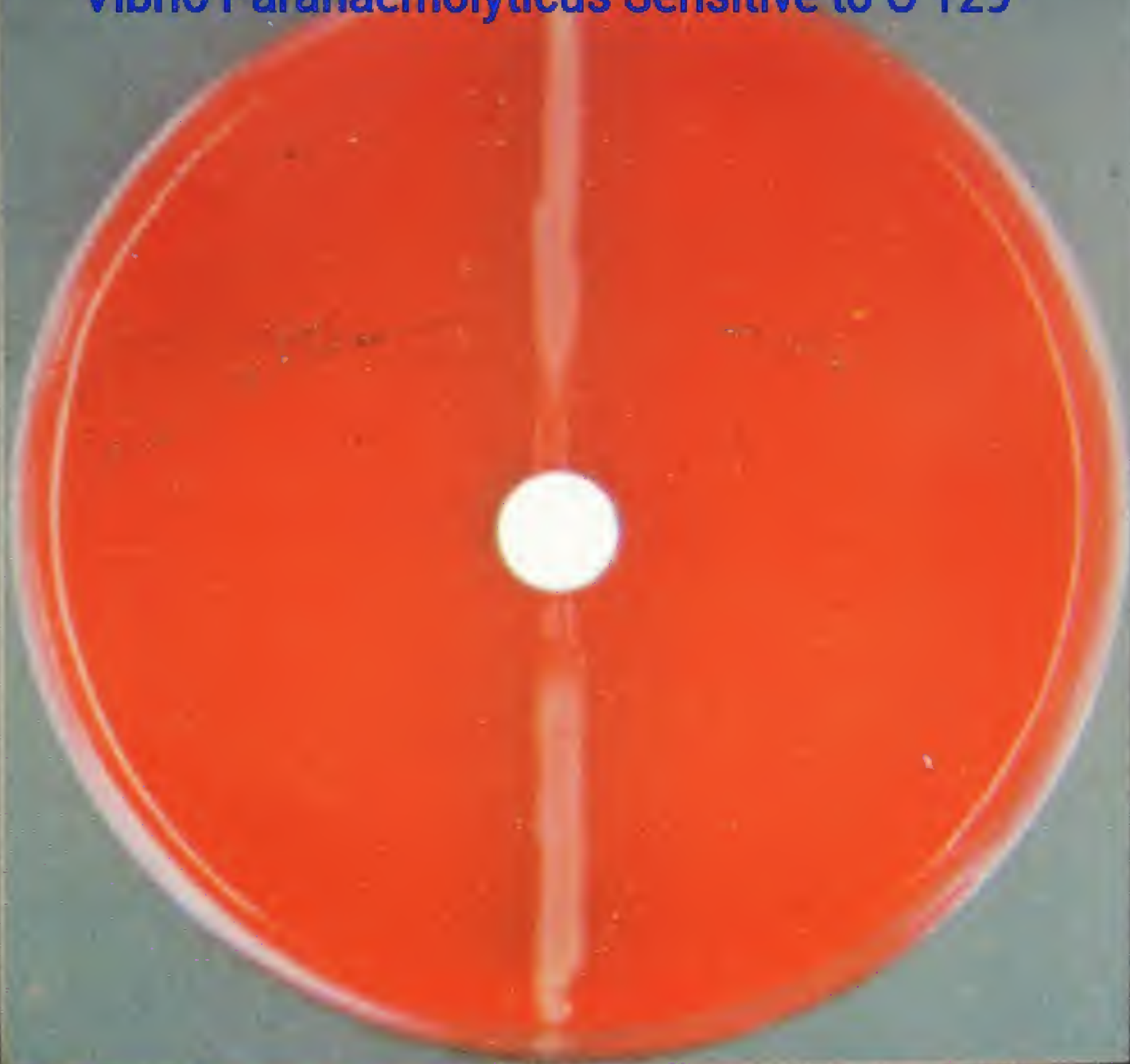




[ Sensitivity to Nalidixic acid ]  
-ve → due to absence of inhibition Zones  
around Central disk Containing Nalidixic acid  
e.g → C.lari

[ Sensitivity to O129 ]

*Vibrio Parahaemolyticus* Sensitive to O 129



[ sensitivity to O129 ]

*Aeromonas hydrophila* resist O129







### [ Optochin test ]

*S. pneumoniae* Sensitive to optochin So , When inoculated on Blood agar with optochin Containing disks not grow around optochin

## [ Sensitivity to Optochin ]

Streptococcus resist optochin SO , grow around  
disks Containing Optochin in Blood agar







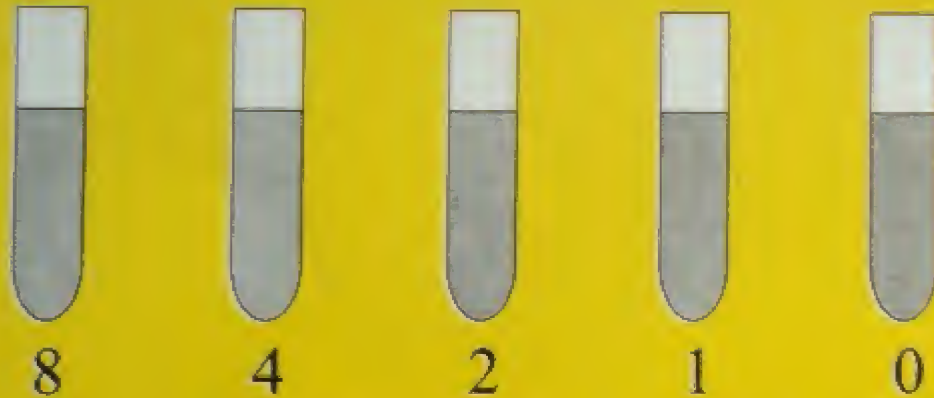
[ gamma phage typing of B. anthracis ]  
Blood agar + B. anthracis +1 drop of Gamma phage  
→ lysis of B. anthracis



# [ Determination of MIC to tetracycline for a tested MO ]

## Disk Diffusion Test

### Determination of MIC

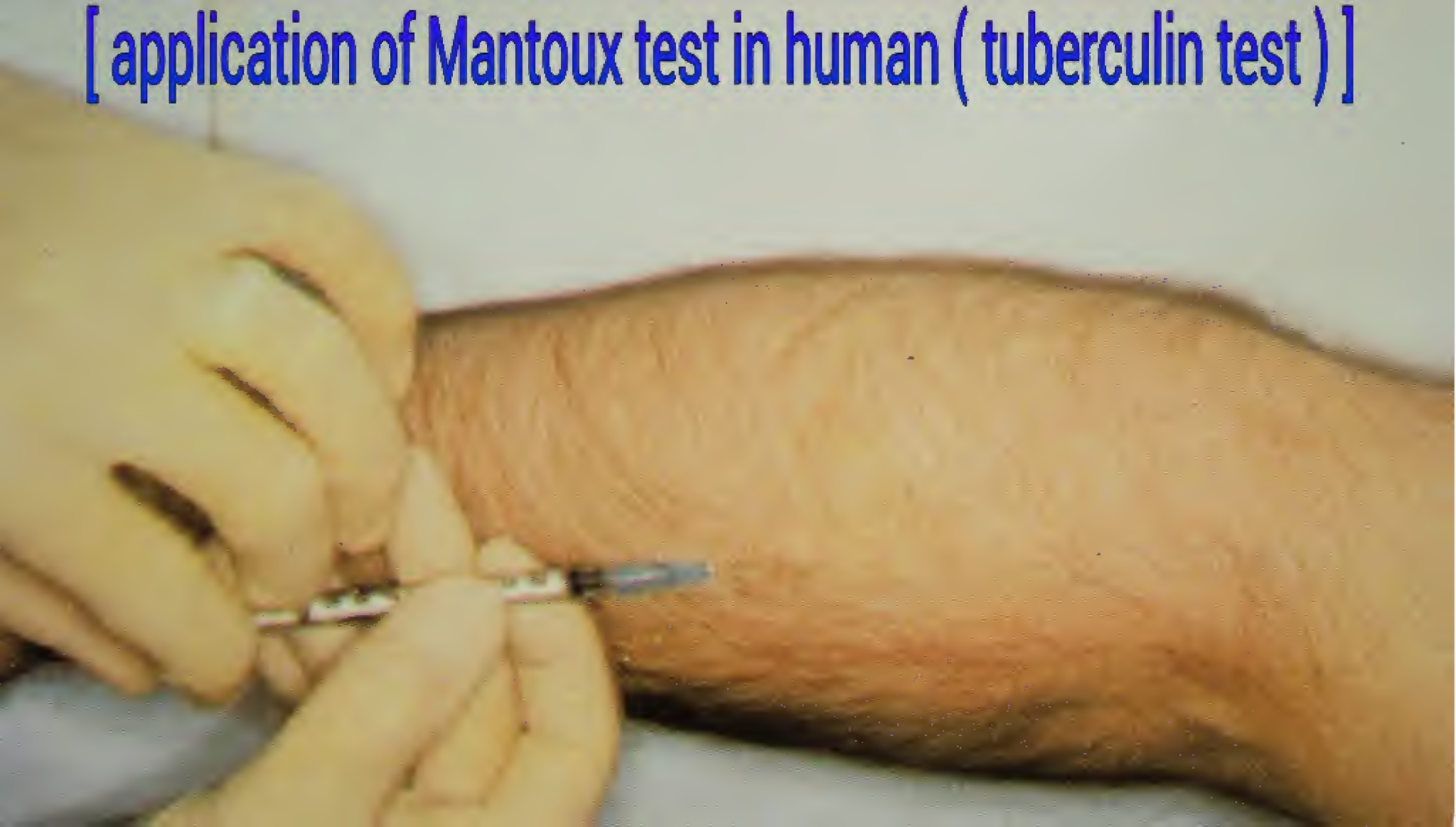


Tetracycline ( $\mu\text{g/ml}$ )

MIC = 2  $\mu\text{g/ml}$



[ application of Mantoux test in human ( tuberculin test ) ]



[ positive result of tuberculin test ]  
due to PPD injection

1 cm





## [ Aesculin hydrolysis test ]

MO hydrolyse Aesculin into aesculetin and glucose

Aesculetin + ferric Citrate  $\rightarrow$  dark brown phenolic Complex

un inoculated medium

+ve *Enterococcus faecalis*



# [ CHO fermentation test ]

Bromothymol blue as PH indicator

glucose fermented and give acid Or acid and gas

acid detected by indicator by Changing Colour into yellow

gas detected by Derham's tube

Un inoculated  
medium

acid

Acid and gas

negative due to  
growth of bacteria





• [ ONPG test ]

O-NitroPhenyl Beta D-galactopyranoside hydrolysed by Beta Galactosidase enzyme into O-Nitrophenol which is Yellow Colour and Galactose

Un inoculated

positive late  
lactose fermenter

Negative



bromothymol blue indicator

# [ OF test ]

oxidation-fermentation test

glucose aerobically → oxidation

anaerobically → fermentation [ acid or acid and gas ]

indicator → Yellow [ oxidation or fermentation ]

blue [ utilization of Peptone and ammonia  
production

un inoculated

Oxidation

fermentation

Gas production

fermentation

Negative  
alcaligenes

1 2 3 4 5 6 7 8 9

## [ Oxidase test ]



The diagram shows two vertical test strips. The left strip is colorless and labeled '- Ve'. The right strip has a blue spot at the top and is labeled '+ ve'.

- Ve

+ ve

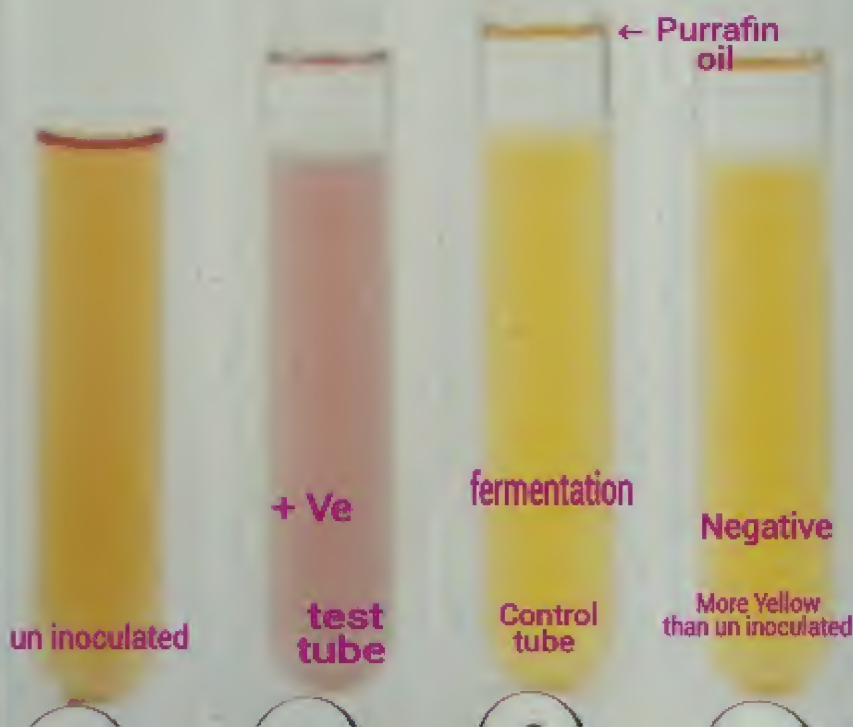
Korack's reagent act as artificial electron acceptor in presence of Cytochrome Oxidase in the suspected Colony in addition to atmospheric  $O_2 \rightarrow$  oxidation of the reagent and produce indo Phenol blue [ blue ]  
if not present  $\rightarrow$  Colourless

## [ Decarboxylase test ]

Decarboxylation of aa lead to formation of amines make the PH alkaline

PH indicator → Bromocresol purple  
violet Colour in alkaline PH  
yellow Colour in acid PH

media used → Moller's medium





[ Simmons Citrate agar ]

Citrate utilized producing alkaline PH  
detected by Bromothymol blue → [blue]

un  
inoculated

**+ ve**    **- ve**





[ Hippurate hydrolysis test ]

Sodium hippurate hydrolysed by hippurate hydrolase

into benzoic acid and glycine

glycine + Nihhydrin Solution →

Blue Coloured Complex

- ve

+ ve




## [ Malonate test ]



Malonate is utilised producing  
alkaline PH Which detected by  
bromothymol blue → [ blue ]







[ Urease test ]  
urea utilised by Urease enzyme  
producing ammonia pink Colour  
with phenol red indicator



un  
inoculated

+ve

-ve

1

2

3



### [ Indole test ]

tryptophane hydrolysed by tryptophanase  
giving indole which produce red ring with  
Kovac's reagent

un  
Inoculated

+ Ve

- Ve

1

2

3



### [ Methyl red test ]

glucose utilised giving large Amount of acid  
making PH = 4.4 giving red Colour detected  
by methyl red indicator

un  
inoculated

+ Ve

-Ve

1

2

3





[ Voges Proskauer test ]

glucose utilised giving acid and acetyls  
Methyl Carbinol producing red Colour With  
alpha naphthol KOH

un  
inoculated

+ ve

- ve

1

2

3

# [ Phenylalanine Deaminase test ]

phenylalanine deaminated giving

phenylpyruvic acid +  $\text{FeCl}_3 \rightarrow$  green Colour

un  
inoculated

+ ve

- ve

1

2

3

*Slant*

[ lysine iron agar ]

glucose & iron & lysine

iron +  $H_2S$  → Iron sulphide [Black]

glucose decarboxylated giving Alkaline PH

detected BY Bromocresol Purple and Cresol red  
as Violet or purple Colour in Slant

glucose → fermented giving acid [Yellow] in Butt

UP  
inoculated

Alkaline /  $H_2S$


alkaline /  $H_2S$

1

2

3





[ Meta bisulphite reduction ]  
 $\text{iron} + \text{H}_2\text{S} \rightarrow \text{iron Sulphide [Black]}$   
Microaerophilic MO

+ ve

- ve

un  
Inoculated

# [ Proteolysis of loeffler's Serum Slopes ]

determine Organisms  
able to breakdown of  
Serum protein

L.M.B

+ ve

- ve

1

2

[ tube Coagulase test ]  
determine if Organism Can  
Clot Plasma by Coagulase

0.5 ml Citrated Human Plasma + 0.5 ml Growth  
incubated at 37°C for 4-24 hrs

+ve  
clot



-ve  
No Clot





# [ Motility test ]

## Soft agar [ Semisolid media ]

un  
Inoculated

Motile

Non motile

1

2

3

4